Workshop on Safeguards for Planned Introduction of Transgenic Oilseed Crucifers

Proceedings

9 October 1990

Cornell University, Ithaca, NY 14853

United States Department of Agriculture
Animal and Plant Health Inspection Service

Edited by: Sally L. McCammon and Sue G. Dwyer

CONTENTS

INTRODUCTION

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has received applications for field testing of transgenic plants on a performance evaluation scale and/or under unconfined conditions. There will also be requests for exemption from the USDA regulatory review process based upon data and experience accumulated from small-scale field tests that have been done as part of the commercialization process. The evaluations of these applications will focus specifically on environmental issues, especially those that may be present in the United States with transgenic plants that can be wind or insect pollinated. The scientific principles used for evaluating transgenic crop plants will be based on the experience gained from traditional breeding.

To identify the appropriate issues to be addressed in analysis of field tests or exemption requests and also areas of uncertainty. USDA-APHIS has sponsored or is planning several conferences and workshops. The Workshop on Safeguards for Planned Introduction of Transgenic Oilseed Crucifers was held for one day in conjunction with the Sixth Crucifer Genetics Workshop, held at Cornell University on October 6 through 9, 1990. The other workshops will include corn/wheat in December of 1990, potato in August of 1991, and rice in the spring of 1992.

The 23 invited panel members of the oilseed crucifers workshop represent several areas of expertise including agronomy, behavioral biology, cell biology, ecology, entomology, genetic resources, molecular biology, plant breeding, plant pathology, pollination biology, seed physiology, and weed biology. They included representatives from academia, government, industry, and public interest groups. In addition, almost seventy observers from countries including France, Belgium, Canada, Thailand, the United Kingdom, and Japan were present.

The scientific discussion during the workshop was for the following purposes:

1. to identify the potential for gene movement to wild relatives and/or non-engineered oilseed crucifer cultivars,
2. to determine the possible negative or neutral consequences of gene transfer and/or expression from oilseed crucifers on agriculture and the environment, and
3. to recommend specific physical, temporal, or biological safeguards for such consequences, if appropriate.

A partial transcript of the workshop was made.

ACKNOWLEDGMENTS

The Animal and Plant Health Inspection Service gratefully acknowledges Stephen Kresovich and James R. McFerson for organizing the Sixth Crucifer Genetics Workshop and their enthusiasm over including the Workshop on Safeguards for Planned Introduction of Transgenic Oilseed Crucifers. Their support made this workshop possible. The beautiful setting at Cornell University and the competence and helpfulness of the Cornell Conference Center were appreciated.

The dynamic discussion led by Dr. Robert M. Goodman as chairman, the executive summary provided by Anne Simon Moffat, and the excellent discussions and papers provided by the panelists made the workshop of special value.

Panelists

Dr. Robert Goodman, *Chair*
Russell Laboratories
The University of Wisconsin
1630 Linden Drive
Madison, WI 53706
USA

Dr. David Astley
AFRC, Inst. of Hort. Res.
Wellesbourne, Warwick CV35 9EF
UK

Dr. Robert Bernatzky
Dept. of Plant and Soil Sci.
Massachusetts Agr. Exp. Sta.
Amherst, MA 01003
USA

Dr. Wally Beversdorf
Crop Sci. Dept.
Univ. of Guelph
Guelph, ON N1G 2W1
Canada

Dr. Michael Clegg (invited observer)
Dept. of Botany & Plant Sci.
Univ. of California
Riverside, CA 92521-0124
USA

Ms. Abby Dilley (invited observer)
Keystone Center
Keystone, CO
USA

Dr. Keith R. Downey
Agr. Canada Res. Stn.
107 Science Crescent
Saskatoon, SK S7N 0XX2
Canada

Dr. Stephen Gleddie
Agriculture Canada
Plant Research Center
Ottawa, Ontario
Canada K1A 0C6

Dr. Rebecca Goldberg
Environmental Defense Fund
257 Park Ave. S.
New York, NY 10010
USA

Dr. Willy de Greef
Plant Genetic Systems
Jozef Plateaustraat 22-B9000
Ghent
Belgium

Dr. Ralph W.F. Hardy
Boyce Thompson Inst.
Cornell Univ.
Ithaca, NY 14853
USA

Dr. Kathleen H. Keeler
Dept. of Biol. Sci.
Univ. of Nebraska
Lincoln, NE 68588
USA

Dr. Steven Knapp
Oregon State Univ.
Dept. of Crop Sci.
Corvallis, OR 97331
USA

Dr. Robin Manassee
Dept. of Zoology
Univ. of Washington
Seattle, WA 91895
USA

Dr. Sally L. McCammon
USDA-APHIS
6505 Belcrest Rd.
Federal Bldg. Rm. 848
Hyattsville, MD 20782
USA

Dr. Martha Mutschler
Dept. of Plant Breeding
and Biometry
Cornell Univ.
Ithaca, NY 14853
USA

Dr. Thomas C. Osborn
Dept. of Agronomy
Univ. of Wisconsin
1575 Linden Drive
Madison, WI 53706
USA

Dr. David Pimental
Dept. of Entomology
6126 Comstock Hall
Cornell Univ.
Ithaca, NY 14853
USA

Dr. Jane Rissler
National Wildlife Fed.
1412 16th St., NW
Washington, DC 20036-2266
USA

Dr. Mattie Sovero
American Pedigreed Seed Co.
Calgene, Inc.
7664 Moore Rd.
Memphis, TN 38120
USA

Dr. M.S. Swaminathan
Centre for Res. on Sustainable
Agr. and Rural Development
14, II Main Road
Kottur Gardens, Kotturppuram
Madras 600 085
India

Dr. James T. Watkins
Sakata Seed America, Inc.
PO Box 6007
Salinas, CA 97912-6007
USA

Dr. Paul Williams
Univ. of Wisconsin-Madison
Dept. of Plant Pathology
1630 Linden Drive
Madison, WI 53706
USA

Executive Summary

Workshop on Safeguards for Planned Introduction of Transgenic Oilseed Crucifers

Ithaca, NY Oct. 9, 1990

Introduction

The anticipated large scale, planned introductions of transgenic, oilseed crucifers (*Brassica* sp.) offer opportunities and challenges not dealt with before in modern, agricultural biotechnology.

Within the last few years, select oilseed crucifers, in particular the oilseed rape known as canola, have become crops of enormous agricultural potential. This crop's level of saturated fatty acids is the lowest of all vegetable oils, with a large fraction of its unsaturated fatty acids consisting of monounsaturated fatty acids; it yields more oil per acre than soybean; and, as a cool season annual, is an attractive winter crop in the mid-south and, in more northerly areas, is an attractive spring-sown crop.

Yet, despite these attractive qualities, brassica oilseeds have distinguishing features that suggest that larger-scale field trials of transgenic plants must be preceded by review. Unlike other transgenic species currently being considered for field trials in the United States, such as tobacco and tomato, many brassicas outcross readily, and they grow in proximity to a number of cross-hybridizing wild, weedy relatives.

Therefore, introduction of transgenic brassica species into the field should include an assessment of the potential for inadvertent transfer and expression of recombinant genes into the same or related species, and the possible risks associated with such transfer. Moreover, identification of such biosafety issues should be made in advance of the planned introductions, if public trust is to be maintained and to avoid potential problems.

As an early step toward dealing with these issues, a conference on "Safeguards for the Planned Introduction of Transgenic Oilseed Crucifers" was held on October 9, 1990 at Cornell University, Ithaca, N.Y., in conjunction with the Sixth Crucifer Genetics Workshop.

The conference sought to:

- identify the potential for gene movement to wild relatives and/or non-engineered oilseed crucifer cultivars,
- determine the possible negative or neutral consequences of gene transfer and/or expression from oilseed crucifers on agriculture and the environment, and
- recommend specific physical, temporal or biological safeguards for such consequences, if appropriate.

The conference format included formal presentations followed by general questions and discussion. The conference chairman, Dr. Robert Goodman, guided the group to consensus on some, but not all, issues.

Because of worldwide interest in the oilseed crucifers, the workshop drew about 70 participants, including substantial numbers from France, Thailand, Belgium and Canada. Scientists from Belgium and Canada discussed data on field trials of transgenic oilseeds made during the past few years. There was a strong sense of international cooperation, which included efforts to share data and harmonize approaches. Overall, the participants were divided among industry, academe, government, and public interest groups, and included laboratory and field researchers, legal specialists, independent agricultural consultants, government regulators and environmentalists. Moreover, the conference was characterized by an expanded dialogue between researchers with basic and applied interests. During the opening session on gene transfer, for example, biologists developing mathematical models, experts on pesticide applications, and scientists studying honeybee behavior shared their very different perspectives. Such open interchange allowed the group to offer suggestions on the management of planned field trials of select transgenic brassica species. Moreover, the wide-ranging discussion allowed the expression of a great variety of individual concerns. Thus, this summary appears conservative compared with the tone of the workshop as it distills and focusses on these concerns.

Part I Gene Transfer

Modern analyses of gene transfer benefit from decades of experience in developing pedigreed seed lines. In the course of building such seed lines, breeders had to establish field protocols that took into account the patterns of pollen dispersal and intra- or interspecific crossing, in order to minimize contamination of the desired gene pool. Techniques were developed for monitoring pollen dispersal, fertilization and the development of viable seed. This methodology, originally developed to measure gene transfer and assure good seed yield in classical plant breeding programs, has offered a valuable tool to assess gene transfer from transgenic species.

Several research groups, in Belgium, Canada, France and the United Kingdom have already completed field trials that use such techniques to quantify gene transfer between transgenic brassica species.

During the 1970's, before the era of transgenic crops, R. Keith Downey, of the Agriculture Canada Research Station, Saskatoon, set an early standard for assessing gene transfer on the major, commercial oilseeds. He determined that the level of pollen contamination from commercial rapeseed fields onto 46 meter square isolation plots, located 46, 137, and 366 meters distant was 2.1, 1.1, and 0.6 percent, respectively, for the self-fertile *B. napus* species. In parallel experiments, the pollen of the self-incompatible *B. campestris* (aka *B. rapa*) traveled further, with contamination levels of 8.5, 5.8, and 3.7 percent. No border effects were detected, nor was the level of contamination affected by the orientation of the isolation blocks to the source of contamination.

A conclusion from these studies was that although a large fraction of pollen falls to the ground within a few meters, under favorable conditions pollen can move long distances by insect and/or wind transportation.

Studies of pollen movement alone, however, offer only a partial picture of gene transfer. Gene transfer can be assessed more accurately by considering the series of hurdles pollen must pass to successfully transmit genetic material, including: travel of pollen to a flower; effective fertilization; the production of viable seed; the resulting plant reaching reproductive age, and, finally, being fertile.

During the late 1980's, Downey probed gene transfer in brassica species in this fuller context. Reciprocal interspecific crosses between commercial oilseed crucifer species, *B. napus*, *B. juncea* were easily achieved in the greenhouse and under field conditions. This suggested gene transfer is possible among these species of crucifer. Tolerance to an herbicide was the marker in these studies.

Of special interest were interspecific crosses with the important weed, wild mustard (*Sinapis arvensis*, aka *B. kaber*). When this species was reciprocally crossed with *B. campestris*, and *B. napus*, no hybrid seeds were obtained, even though pollinations were made on emasculated buds in favorable greenhouse conditions. Also, no hybrid was obtained in the cross *S. arvensis* x *B. juncea*, although the reciprocal cross produced 2.5 hybrid seeds per 100 bud pollinations. But the F1 plants of this cross were largely male sterile and set no seed on self or open pollination. Moreover, when the F1 plants were back-crossed to *B. juncea*, only one non-viable seed was produced in 1,003 pollinations. Also, in 881 backcrosses to *S. arvensis*, one seed was obtained, but the plant resulting from this seed was sterile.

Downey concluded from these trials that, "gene transfer to *S. arvensis* (wild mustard) from the three major crops (*B. napus*, *B. campestris*, and *B. juncea*) was not achieved even under the most favorable conditions, and no hybrids were identified from natural crossings of the species when they were co-cultivated in field plots over a three-year period."

However, when *B. nigra* and *S. arvensis* were crossed, hybrid seed were readily obtained when *B. nigra* was the female. To a lesser degree, when the reciprocal crosses were made, hybrid seeds were readily obtained. This suggests that *B. nigra* may serve as a bridge between some cultivated and weedy brassica species.

Downey discounted the possibility that *B. nigra* serves as such a bridge in western Canada, noting that *B. nigra* is not a common weed, is rarely found in association with cultivated *Brassica* species and is, therefore, not considered a serious threat. In eastern Canada, where *B. nigra* is found in the Great Lakes region, it has a small distribution on waste sites and is rarely found with cultivated fields.

Another recent effort to probe gene transfer in brassicas has been funded by the European Community and the United Kingdom at field sites in France, Belgium and the United Kingdom, and was reported by Willy de Greef, of Plant Genetic Systems (PGS), Belgium. In 1989, circular fields were set out, 100 meters in diameter, with a three meter diameter source of brassicas plants transformed for tolerance to the herbicide glufosinate. The researchers found a massive drop in pollen density close to the source, with all outcrosses found 12 meters or less from the source of pollen. They also identified several flaws in the experimental design, which are being corrected in preparation for the next round of experiments. In particular, the pollen source is being expanded to nine meters in diameter and sampling sites are being moved closer to the source of pollen. Additional strong, selectable markers that allow easy identification of low frequency events are still being sought. de Greef stressed the need to determine and study the key environmental parameters that are most critical to determining pollen transfer among brassicas and related genera. These parameters may include richness of pollen source, weather, efficiency of insect pollinators, and others.

Yet another approach to assessing gene transfer, offered by Robin Manasse of the University of Washington, is mathematical modeling for prediction of gene spread. Such studies have helped to verify that pollen travel falls off exponentially from the source.

An advantage of such a modeling strategy is that it allows improved understanding of pattern and dynamics. It also offers a strategy for going beyond the restricted results of individual studies. But this method for describing gene flow, which begins with painstaking field work and extends the data via mathematical approaches, is still very much at the initiation of a steep learning curve. The initial modeling equations considered only a homogenous environment, which bears limited resemblance to the natural world, but such studies are now being expanded to consider heterogenous situations. A major challenge for development of a more complete model of gene transfer is deciding what aspects of the environment, such as the effect of surrounding vegetation on pollinator behavior, or weather, are most critical to the model. These parameters then need to be studied on their own, in some detail, before they can be usefully incorporated into a model of gene transfer.

These various approaches to the study of gene transfer will eventually change the discipline from a descriptive science, to a manipulative one, to a more predictive one. However, some key issues must be dealt with before this goal can be realized.

The need to study gene transfer under relevant conditions was mentioned repeatedly. In particular, there is a need to better understand those key contextual factors such as weather, pollinator behavior, local topography or cropping patterns, that most influence gene flow among brassica species. They must be identified first, and then studied.

It was also suggested that a better understanding is needed of the importance of scale in experimental design. In particular, the factors determining the scale an experiment must be carried out on, to yield meaningful data, are unclear. The problem is especially timely as researchers move from small scale to larger scale field trials. Keith Downey articulated this concern in his comment that, "...there is an area of uncertainty as to the controls that may be imposed as we move from testing in an isolated transgenic block to cooperative yield trials or to field-scale multiplications for pilot plant extraction and product evaluation."

Most important, studies of gene transfer among brassicas have an added complexity because these species outcross easily. In the past, most studies of gene transfer focused on gene exchange among species within the same genus. Now, with the brassicas as crops of interest, researchers must survey gene exchange among species of related but different genera (e.g. *Sinapis*, *Raphanus* and *Eruca*). It was agreed by most that with larger-scale field trials, gene transfer out of the test species will happen.

There was consensus that the study of gene transfer in oilseeds should not focus on whether or not genes from transgenic species would move out; but rather, the study should focus on the conditions under which transfer and expression occur, and what are the consequences of these events.

Summary Section I - Gene Transfer

- Protocols that have been developed to establish pedigreed seed lines can be adapted to assess gene transfer in transgenic species.
- Field studies of gene transfer are being extended through mathematical models.
- The oilseed crucifers are highly outcrossing crops.
- In larger-scale field trials of transgenic brassica species, gene transfer out of the test species to wild or weedy relatives is likely to happen.

Part II Consequences of Gene Transfer

The classical approach toward risk assessment involves consideration of risk as the product of the probability of hazard times the probability of exposure (r=h*e). As applied to evaluation of field introductions of transgenic oilseed crucifers, risk is equal to the product of the probability that a gene will cause problems (h), and the probability that a gene will escape from intended locations to sites where it can cause a problem (e). This approach is a favored first step to analyzing the consequences associated with unintended gene transfer since it uses established methods, is potentially quantitative, and assumes that a complex problem can be broken into simple segments.

In analyzing the r=h*e equation, the exposure component was judged to be the more manageable variable. For transgenic crucifers used only for their vegetative parts, exposure and gene transfer could be prevented by introducing systems that could prevent production of fertile flowers. But for transgenic crucifers valued for their seeds, such efforts would be worthless. Still, exposure can be managed somewhat by releasing plants that can be cheaply and, most important, efficiently eliminated to prevent carryover growth the next growing season.

Most of the discussion, however, focused on analysis of the "h" component of the equation. The possibilities for "hazard" that received the most attention were weediness, herbicide tolerance, disease resistance, and insect resistance. Loss of diversity and potential contamination of the gene pool were also mentioned as possible hazards.

A risk analysis of those genes that determine such traits requires knowledge of the genes in question, their origin, how the gene products behave, their mode of action (e.g. interaction with genetic regulators), estimation of their behavior if escaped and, most important, assessment of fitness within a range of environments. At the next level, a description of the species that may be affected by such genes, how these species respond, and the resulting environmental changes, including those affected by scale, may be needed. Unfortunately, data are scarce or absent for many of these concerns, especially those that are ecological in character. Most conferees agreed with the advice of Robert Bernatzky, Massachusetts Agricultural Experiment Station, Amherst, that, "It would be prudent to invest in serious impact studies on the effects such (select transgenic) genes would have on competitive ability prior to large scale growing of transgenic plants." Assessments of specific transgenes in particular plants, in select geographic settings, were called for repeatedly.

Moreover, the value of historical experimental data was stressed. It was noted that information about the transfer of select traits, such as seed quality and pest resistance, from traditionally bred crops to wild species, may be available in existing plant populations in Canada, where significant changes in the types of rapeseed grown have occurred over the years.

Several ideas were offered to establish a theoretical framework that could guide new field experiments. Such a framework might focus efforts and give increased meaning to a series of costly empirical trials.

Kathleen Keeler, University of Nebraska, called attention to an ecological principle of population regulation which, she says, "...has worrisome implications for the release of transgenic crops." Keeler says that many of the genes that can be effectively transferred to plants, and that show economic

potential, are genes which offer biotic resistance, that is, resistance to other organisms, including herbivores or pathogens. Ecologists agree that such biotic interactions can and sometimes do limit plant numbers. If biotic interactions—competitors, pathogens or herbivores—keep a plant relatively rare, and it becomes resistant to such biotic interactions, more seeds will be set and the numbers might rise to where it becomes a weed. Therefore, changes in biotic interactions can cause weed problems. This basic principle of population control suggests that all transgenes that alter a plant's biotic interactions have the potential for increasing its weediness. This says Keeler, "...means that transgenes for herbicide resistance, insect resistance, and disease resistance all have the potential for causing weed problem."

Another effort to establish a framework for judging the outcome of the r=h*e equation involves classifying the risk of transgenes into several categories: high fitness, for those genes that endow a plant with some broad-based defensive quality, such as insect or disease resistance, and would persist a long time; moderate fitness, for those genes that endow a plant with a quality or value in a special setting, such as herbicide tolerance or altered biochemical composition; and low fitness, for those genes, such as male sterility, that would handicap a plant and have low persistence in the gene pool. Moreover, consideration must be given to recessive genes that may be hidden in a population indefinitely, but not permanently.

However, despite the desire to develop a scheme that could impose some order on risk analysis, there was general agreement that the process was fraught with pitfalls. Said Thomas Mitchell-Olds, University of Montana, "We can generalize, but the specifics matter. If asked to rank the issues, the secondary or unintended effects may often prove those of most concern because their effects will be unexpected." And Keeler noted, "it is impossible to give a blanket exemption for any trait, however, because somewhere, there is an environment in which this (trans)gene raises the fitness of a weed...Nevertheless, she added, "many genes can be agreed to be benign in real world environments, especially after a few experiments on the behavior of the transgenic phenotype."

With this is mind, some agreement was reached on the relative risks of various transgenes. There was no agreement that a single trait presented the greatest risk. Rather, there was agreement that a cluster of traits, including the weediness of plants that could receive a transgene, as well as transgenes for disease, insect, and herbicide resistance, merited special attention.

Possibly the most frequently voiced concern, although it was far from unanimous, was that the field production of transgenic crucifers might result in the creation of a new serious weed or increase the aggressiveness of existing weeds, that is, those plants that interfere with human activity. A third possibility is that cultivated transgenic oilseeds might turn into weeds themselves. This is a special concern in Europe, where volunteer, winter *B. napus* is becoming increasingly persistent along roadside medians. In the United States, *B. rapa* is also a common weed and *B. napus* is becoming so in broad-leaved crops in the northwest.

A suggested first step in analysis of the risk of weediness is to assess whether there is a manageable or acceptable level of weediness. Put another way, does a particular transgene change a brassica plant to create a weed worse than one that already exists? This analysis is not easy, says Kathleen Keeler, since, "It may depend on the (context of the) situation." She considers the following example. Weedy brassica populations with naturally occurring tolerance to the herbicide triazine exist. A crop with these same exact herbicide resistances, growing in the area where naturally occurring resistant brassica populations are found, will pose no additional problem to agriculture. However, in areas where weedy brassica populations occur, but not triazine resistant ones, the appearance of triazine-tolerant, weedy brassicas could pose a hazard to agriculture, if triazines are used.

Other examples were given of how the context of a situation can affect a plant's ability to become a weed. For example, weeds prosper in disturbed environments, such as roadsides, arable fields and footpaths. Therefore, the tendency of a transgenic crucifer to become weedy may depend on the availability of such disturbed settings.

However, in the absence of a theoretical framework for describing how context could affect a plant's proclivity to weediness, a series of experiments, specific to organism and situation, was recommended. A preeminent contextual issue is whether wild relatives grow in the range where the transgenic crop will grow. The formulation of ecological maps that describe the range of such relatives was recommended.

Several other generalizations emerged from the discussion on weediness. For example, of the limited evidence collected to date, no genetically modified organism has been known to become weedy by gaining traits such as herbicide or pest resistance. Also, since most domesticated hybrids are less vigorous than the wild type (the emphasis in plant breeding has been on those traits that coincidentally reduce fitness in the wild), such crops are unlikely to degenerate into weeds following further genetic manipulations. Finally, it was noted that most weeds have a mix of traits that define their aggressiveness. On the other hand, most transgenic plants have only one or a few modified traits. Still, there is the possibility that the genetic modification of only a few, select traits, such as seed dormancy, could enhance a plant's ability to become a weed.

The development in some weed species of cross resistance, where a single genetic change results in tolerance to more than one herbicide, or multiple resistance, where two or more distinct genetic changes result in tolerance to more than one herbicide, were other frequently mentioned possible hazards of field scale trials of transgenic oilseeds. David Astley, Horticultural Research International, Warwick, United Kingdom, noted that a variety of commercial companies are developing cultivars of the same crop that have insensitivity to different herbicides. Over time, this effort could yield an additive effect, leading to weed populations with a multiplicity of resistances. Several speakers were uncertain as to whether select herbicide resistances conferred greater or lesser fitness on a plant. Again, experimental trials, specific to trait, crop, and environment, were advised.

There was a lack of consensus on the importance of insect resistance as a hazard. Insect resistance is rarely a single trait and, therefore, blanket generalizations are difficult to make. Willy de Greef noted that no single strain of *Bacillus thuringiensis* (Bt) can affect a whole order of insects. Moreover, so far, no research group has attempted to insert various Bt genes into a single plant variety because it would be a huge metabolic drain on the plant. On the other hand, some participants agreed with Kathleen Keeler's assessment that all transgenes that alter biotic interactions have a potential for being hazardous.

The judgment on whether disease resistance presents a serious potential hazard was similarly divided. Like insect resistance, disease resistance may not be a single trait, and subtleties about the mechanisms of disease resistance can determine the scope of the hazard. Yet, like insect resistance, disease resistance is a biotic trait and altering it may release a plant from natural checks. It should be noted, however, that plant breeders have been transferring disease resistance genes—from wild relatives to cultivated crop species—for generations with no known adverse affects.

Most changes in plant composition, such as lipid composition or quality, and protein or fiber composition, were judged low risk, with one exception. Such changes might alter a plant's palatability to pests and this, in turn, could alter its fitness. Again, specific tests were called for.

A number of other risks were of particular concern to some speakers. For example, David Astley stressed that increased use of transgenes in the field should encourage those who maintain and monitor gene banks to take special precautions that such banks are not contaminated. Rebecca Goldburg, of the Environmental Defense Fund, said the development of crucifers that tolerate and therefore encourage use of environmentally damaging herbicides, such as atrazine, would be undesirable. Jane Rissler, National Wildlife Federation, suggested that a general hazard associated with the widespread use of transgenic plants is reduced diversity. However, others noted that management procedures, which encourage variability in planting schemes, are available for minimizing this potential problem. On a related subject, Ralph W.F. Hardy, of the Boyce Thompson Institute for Plant Research, Ithaca, New York, said that various risks, such as increased vulnerability to pathogens, arise in a few cases when highly advantageous genotypes are used continuously and exclusively, such as Texas male sterile cytoplasm corn. But, he suggested, that coordinated efforts to maintain non-exclusivity in the use of advantageous genotypes can avoid such problems. "We must be insightful at the producer level and implement the appropriate management procedures," he said.

Summary Section II - Consequences

- The classical approach to risk assessment involves evaluating the product of likelihood of hazard times likelihood of exposure (r-h*e).
- Theoretical guidelines are available for designing field experiments to test the consequences of gene transfer.
- The hazard mentioned most often was weediness. However, there was no consensus on a single, preeminent hazard. Others receiving special attention included herbicide, disease and insect resistance, loss of diversity and potential contamination of gene banks.
- Assessments of gene transfer of specific transgenes, in particular species, in select geographic settings, were recommended. Such assessments will require several years of experiments.

Part III - Safeguards

Society may derive the benefits of genetic engineering, including some that carry high risks, by implementing a variety of techniques for managing hazards associated with gene transfer. Such transfer is likely in developmental scale field trials or after commercialization. Experiments on pollen dispersal, which have been carried out over the last few decades, as well as experience developing purebred seedlines, offer strategies for monitoring gene transfer.

Keith Downey noted that, in Canada, the current regulations for small plot testing of transgenic brassica oilseeds appear adequate. However, it is unclear whether such regulations can be easily scaled up, as trials move from isolated test blocks to larger fields. "From our experience," Downey says, "we would recommend that each introduced gene should have its own risk assessment and, if no hazard is identified within a three year isolation test and evaluation program, the transgenic material should be handled in the same way as we would introduce a mutated strain into the testing and evaluation system."

David Astley, said that in Europe, the recommended isolation distance to maintain the genetic integrity of a brassica seed production crop is 400 meters. An experiment, done under the aegis of the Planned Release of Selected and Modified Organisms (PROSAMO) initiative, to monitor the movement of a known gene from genetically manipulated plants in the center of a 1 hectare field populations of *B. napus*, is currently being analyzed at the Centre for Plant Science Research, United Kingdom.

However, Astley suggested that, "Larger scale experiments without containment will present additional problems including the security of a field area, minimization of the probability of GMO [genetically modified organism] pollen reaching a non-experimental hybridizable plant, unrestrained access to pollinators and wind/freak climatological events. Isolating a GMO experimental plot in a large, crucifer-free area, such as a weed-free barrier crop, with introduced bee hives, would provide the basis for a 'controllable' experiment."

He added that, based on the results of conventional work done to set and maintain seed production standards, "an exclusion zone should extend to a radius of 400 meters. An

ecological survey of the exclusion zone would identify potential problems with crops, weed species and bees from wild hives. The experimental site and 'exclusion zone' will require strict, routine removal of potential pollen recipients, especially *B. napus* and *B. rapa* and wild bee hives."

Many speakers noted that, for now, all such efforts to monitor gene movement in transgenic crucifers are handicapped by a shortage of good marker genes. Genes for herbicide tolerance (e.g. glyphosate, a sulfonylurea, and glufosinate) are the ones most commonly used.

In addition to monitoring gene escape via the exclusive use of field studies, progress is being made in developing prediction techniques that rely on mathematical modeling. Robin Manasse and Peter Kareiva, University of Washington, have used the theories of H. F. Weinberger and N.K. Shigesada to identify select parameters that help describe gene spread over a variety of novel plant spatial distributions. In addition, they have started field experiments to test the predictive powers of their models. They have found that the maximum rate of gene spread is a function of mean gene dispersal distance and the relative fitness of the gene in question, compared to its corresponding allele in a wild population. Manasse says that, "Monte Carlo simulations have shown that within a homogenous environment, Weinberger's equations give a very good prediction of gene spread."

But she also offered two caveats to using this scheme for predicting gene spread. First, determining the relative fitness of a gene or plant is a non-trivial task. Second, the current equations describe gene movement in a homogenous environment, which may not hold in a natural ecosystem. Perfecting such models to accurately describe the realities of the complex, natural world will require much additional work.

The challenge of managing hazards prompted a discussion of both conservative and creative approaches toward the use of safeguards. The most conservative strategy for safeguarding transgenic crop systems is to introduce only those genes and species for which gene flow is acceptable. However, approaches that allow more flexibility were preferred by many of the participants.

For example, in discussing various schemes for safeguarding crop systems including transgenic crucifers, Kathleen Keeler noted that the discipline of "Immunology, needing antibody production but not wanting to introduce live viruses, developed attenuated viruses: the plant equivalent might be male sterile or apomictic cultivars. Breeders, approaching a high risk release might be prudent to add the "Achilles' heel", a control mechanism (preferably a good one and a backup method) as additional methods of control of the released plant."

Several classes of Achilles' heel were discussed. One involves the design of safeguards built into the plant, usually by genetic means. Another involves the use of specific management practices, including specific agronomic practices.

A general safeguard against unwanted gene transfer involves building diversity into the agricultural ecosystem. This may be done by, for example, rotating crops, herbicide classes and barrier crops, and by moving test plot locations. Other agronomic practices that could safeguard against gene transfer include maintaining ample isolation distances and, when available, using significant natural barriers, such as waterways and hills, to isolate crops.

Genetic manipulations that may offer safeguards include designing plants with special nutritional requirements or herbicide sensitivities. In addition, a 'suicide gene' might be designed that could encourage species to self-destruct in defined circumstances.

However, there was some reluctance to rely totally on genetic manipulations to correct failings in safeguards. The value of traditional mechanical and chemical means for managing and safeguarding crop systems was not discounted.

Also, David Astley noted that, in an effort to safeguard against contamination of seed banks, the United Kingdom's Royal Commission on the Release of GMOs to the Environment recommended that, "A viable genetic resource sample of the unmodified cultivar be placed in long-term storage."

Summary Section III - Safeguards

- Established field techniques, as well as more recently developed modeling techniques, can be used to monitor and predict gene transfer. However, such efforts are stymied by the paucity of good marker genes for measuring low frequency events.
- Various safeguards against unwanted gene flow are available. Some rely on established agronomic management practices, such as crop rotation and the use of barrier crops: others rely on genetic changes that place manipulatable "Achilles' heel" into a species.

Workshop Papers

Paper 1

David Astley
Institute of Horticultural Research, United Kingdom

General recommendations on risk assessment and codes of practice for the release of genetically modified organisms (GMOs) have been produced by the Organisation for Economic Cooperation and Development, United States Department of Agriculture and various European governments. National advisory "watchdog" committees have the legislative brief to assess and monitor scientific programmes.

Risk assessment for the use of GMOs is divided into i) a definition of the laboratory work in terms of the species involved, gene identification and DNA sequence, effects on the host phenotype and physiology, and ii) the potential for gene transfer either within or between species, the effect of altered combinations of genetic material on persistence of the GMO in the environment and its competitiveness with crops and wild species, and consequences to other biota and the environment.

Genetic manipulation of oilseed crucifers aims to improve yield and quality, and to confer resistance to pests and diseases and insensitivity to herbicides. The final products of such manipulation are intended for large-scale field production. Therefore, ultimate field assessment must be on a scale conducive to the provision of valid scientific conclusions on the GMO performance and its interaction with other biota and the environment. But the experiment must be under sufficiently close control to guarantee the containment of the transferred gene or novel gene combination. In the UK the PROSAMO (Planned Release of Selected and Modified Organisms) initiative includes a *Brassica napus* program focussing on ii) above.

Literature surveys provide considerable background on the pollination and pollinator biology, and crossability of oilseed crucifers relevant to the transfer of genetic material from a GMO. Oilseed crucifers are visited regularly by insect pollinators irrespective of whether they are self-incompatible, obligate outbreeders (*B. rapa*) or exhibit degrees of autogamy (*B. napus*). Experiments monitoring the movements of bees to and within rape crops have shown that long distances may be travelled to a nectar-rich crop, but once in a crop bees forage within a relatively small area, $10m^2$, tending not to cross field boundaries to other nectar/pollen sources. Bateman (1947) quantified the relationship in *B. rapa* between percentage seed set and distance from the compatible pollen sources as: 60% @ 6m, 13% @ 24m, 6% @ 43m and 1% @ 156m. In Europe the maximum recommended isolation distance to maintain the integrity of a *Brassica* seed production crop is 400m. Complex models on foraging behaviour of pollinators have been produced but still leave many of the points raised in a risk assessment exercise open to interpretation. A PROSAMO experiment to monitor the movement of a known gene from genetically manipulated plants in the centre of a 1ha field population of *B. napus* is being analysed currently in the Centre for Plant Science Research, UK.

If pollen is carried beyond the limits of the experimental field then transfer of genetic material to non-experimental plants is a possibility. Crops and wild species of several genera, including *Brassica, Sinapis, Raphanus* and *Eruca* are potential recipients of pollen from genetically manipulated oilseed crops (*B. napus* and *B. rapa*). However, experimental intergeneric hybridisations have been achieved only after numerous hand pollinations and embryo rescue. Inter-specific crosses in *Brassica* exhibit different levels of success depending on the species combination. The two oilseed species are inter-fertile producing a vigorous sesquidiploid which can stabilize genetically through backcrossing to either parent. Crosses with *B. oleracea* as the maternal parent are possible, but difficult with *B. rapa* and extremely difficult with *B. napus*. However, the degree of success in inter-specific hybridizations varies depending on individual genotypes and environmental conditions making open-field assessment complex. Therefore in terms of successful chance hybridisation by GMO pollen the crosses of highest probability will occur between other brassica crops or volunteer weeds of the two oilseed species. In practical terms the major concern must focus on intra-specific and inter-specific hybridisation of the 2 rape species, *B. napus* and *B. rapa*. *B. rapa* is a common weed in the USA and increasingly volunteer *B. napus* is becoming a problem in broad-leaf crops and field margins. Therefore planning of an experiment will need to take account of such possibilities by incorporating an exclusion zone for potentially inter-fertile material.

For small-scale experiments physical containment is feasible using insect-proof, gauze cages. Bees can be introduced for pollination-monitoring experiments. Following the plant harvest, bees could go through a relatively short quarantine period with an artificial food source providing time for the elimination of GMO pollen from pollen-sacs and body surface. If the GMO plants are seeded the isolation cage site must be monitored for volunteer seedlings in subsequent growing seasons.

Larger-scale experiments without physical containment will present additional problems including the security of a field area, minimization of the probability of GMO pollen reaching a non-experimental hybridisable plant, unrestrained access to pollinators and wind/freak climatological events. Isolating a GMO experimental plot in a large, crucifer-free area, such as a weed-free barrier crop, with introduced bee hives would provide the basis for a "controllable" experiment. Based on seed production standards such an exclusion zone should extend to a radius of 400m. An ecological survey of the exclusion zone would identify potential problems with crops, weed species and bees from wild hives. The experimental site and "exclusion zone" will require strict, routine removal of

potential pollen recipients, especially *B. napus* and *B. rapa* and wild bee hives. Bees from experimental and wild hives, and other pollinating insects will need to be monitored throughout the flowering period of the GMO. Additional studies on the possibilities of pollen transfer by solitary bees, hoverflies and pollen beetles are required.

The barrier crop would limit the transfer of pollen by the wind to non-experimental, crossable plants but would not be effective against the dissemination of plants by freak weather such as hurricanes/tornadoes. Very strong winds could transport bees carrying GMO pollen significant distances. But generally pollinating insects are very sensitive to changes in atmospheric pressures and hence not active during extremes of weather. For each potential experiment site, local weather records should provide a prediction on the probability for the occurrence of hazardous conditions. At the completion of the experiment, plant material should be destroyed, preferably on site, to avoid dissemination of GMO seed, and the test site monitored subsequently for volunteer plants. Such an isolated site could pose security problems.

Both oilseed species are successful weeds. In Europe volunteer *B. napus* is becoming increasingly persistent in other broadleaf crops and roadside verges. It is difficult to predict how the transfer of herbicide insensitivity or disease resistance from a GMO to a weed population would affect the persistence or competitiveness of that population. In Canada weedy *B. rapa* populations which developed maternally inherited insensitivity to atrazine did not spread beyond their original area in 5 years. Insensitivity to atrazine was transferred to cultivars of *B. rapa* and *B. napus* using conventional breeding techniques. However, insensitivity to herbicides based on nuclear genes, and thereby transferrable through pollen, would have a greater potential for spread.

The evolution of cross-resistance to herbicides in some weed species is causing concern. In considering the possibilities of controlling volunteer weeds, research workers should screen any new GMO with herbicides. Marked susceptibilities can be recorded for future use against any volunteer plants. Commercial companies are developing cultivars of the same crop with insensitivity to different herbicides which in the long-term could lead to weed populations with a multiplicity of resistances.

In the transfer of a desired trait into a target genotype there is no guarantee that a linked undesirable characteristic may not also be transferred. The unwanted gene may prove difficult to eliminate. One of the points made by the Royal Commission on the release of GMOs to the environment in the UK is that a viable genetic resource sample of the unmodified cultivar should be placed in long-term storage as a security measure. If necessary, it would be possible to return to the original genotype from the collection.

Genetic resources conservationists are becoming increasingly interested in the collection, conservation and utilisation of wild and weed species. The unwitting transfer of a gene or novel gene combination to a wild/weed taxon could lead to that material being collected and entered into a generic resources collection.

Provided that genetic resource management practices designed to maintain accession integrity during seed regeneration were observed, further dissemination of the gene within the collection would be avoided totally. In-house characterisation of the accession would offer the possibility of gene transfer within the collection and to local crops and weeds. But the major concern would be the global distribution of a GMO-contaminated accession to research workers by a collection curator in total ignorance of the accession's genetic history.

Paper 2

Robert Bernatzky
Department of Plant and Soil Sciences, University of Massachusetts

Oilseed crucifers are highly outcrossing plants. In fact, the diploid oilseed crucifer (*Brassica rapa*) is an obligate outcrosser due to self-incompatibility. The tetraploid *B. napus* is normally self-compatible but does outcross, and self-sterility is actively being sought as a means of hybrid seed production. In a closely related crucifer, *Raphanus sativus* (radish), interpopulation mating rate estimates indicate that gene flow is as high as 3–18% for isolation distances of 100–1000 meters (Ellestrand and Hoffman, 1990). Weedy forms of *B. napus* and *B. campestris* do exist at their centers of origin, and *B. oleracea*, which can cross to both of these species to a limited extent, also has weedy forms. *B. napus* is a natural amphidiploid derived from a cross between *B. campestris* and *B. oleracea* and was likely formed a number of times (Tsunoda et al. 1980). Therefore, the potential for initial gene escape through cross-pollination should be considered as high. The extent in range and abundance of wild and weedy relatives of oilseed crucifers needs to be determined in those areas of the United States where transgenic plants might be grown. Isolation distances for these crops also needs to be determined, but if the rates of interpopulation mating are similar to those of *B. sativus*, then prevention of gene flow by isolation is probably not feasible.

The survival of transgenic material outside of testplots will depend primarily on the adaptive qualities bestowed upon the plants by the introduced genes. Most of the characters that plant breeders select for have little or adverse effects on a plant's ability to survive in the wild. Certain genes, such as those that condition oil quality, may have minor effects on adaptability, whereas those that confer resistance to pests and diseases could provide significant competitive ability to plants in natural populations. Genes that provide tolerance to adverse climatic conditions could also enhance a wild or weedy relative's ability to compete. Besides producing potentially more noxious weeds, this 'adaptive' class of genes could also produce ecological changes such as shifts in biodiversity in natural populations of both plants and pests. It would be prudent to invest in serious impact studies on the effects such genes would have on competitive ability prior to large-scale

growing of transgenic plants. There probably already exists data on the effects traditional plant breeding has had on weedy relatives for genes from natural gene pools that confer resistance to pests or tolerance to the environment. It is in the novel genetic constructs derived artificially or from interkingdom transfers (e.g. Bt toxin) that more data is needed to determine the ecological consequences of gene escape.

The problem with safeguards to prevent or minimize gene escape is that these measures will most likely produce added cost to crop production to the point where the safeguards may cancel the benefits from the new genotypes. Border rows of non-transgenic plants would certainly reduce pollen flow outside of test or commercial plots and this could be tested using easily scored visual genetic markers in model non-transgenic plant populations. Self-compatibility would only increase the proportion of selfed progeny but would have little effect on the physical movement of pollen beyond test plots, that is, the amount of self pollen that falls on a self or neighbor's stigma is the same for self-compatible or self-incompatible plants. Self-compatibility only impacts on the ability of the pollen to achieve self-fertilization. Male-sterility has great potential to prevent gene flow through pollen but the problem lies in the fact that the product is seed and requires pollen. It would be useful to test the utility of interplanting non-transgenic pollen donors among male-sterile plants to determine if comparable levels of seed production still occur without sacrificing too much field space. Tight linkage of the male-sterile gene system to the 'new' gene would be the most effective way of reducing gene flow, even through seeds.

Biological controls may provide some safeguards if gene systems can be developed that affect a plant's ability to survive outside of the field without affecting its performance within. Although such systems may not presently exist, they may take the form of self-destructive gene that is suppressed by another gene in trans. Recombination would tend to break up the association and make the self-destructive gene operable. Another approach might be to link the 'new' gene with a self-destructive gene that could be induced under very specific conditions. A highly specific, non-toxic chemical could be used to induce the destructive system and could be used to control weedy recipients of the 'new' gene. These are of course speculations, but not beyond the realm of molecular biological methods.

References:

Ellstrand, N.C., and C.A. Hoffman. 1990. Hybridization as an avenue of escape for engineered genes. BioScience 40:438–442.

Tsunoda, S., Hinata, K., and C. Gomez-Campo. 1980. Brassica crops and wild allies. Japan Scientific Societies Press, Tokyo, Japan.

Paper 3
Biosafety of Transgenic Oilseed Crucifers

R. K. Downey and D. J. Bing
Agriculture Canada Research Station, Canada

In order to assess the possibility of gene transfer among and between the major oilseed and weedy species of the Cruciferae, one needs to know the normal pattern of pollen dispersal and degree of interspecific crossing that can occur under natural as well as the most favorable artificial conditions. Research on both these questions has been ongoing at the Saskatoon Research Station for a number of years. This work was undertaken to establish regulations concerning suitable isolation distances for the production of pedigreed seed, and more recently in conjunction with the evaluation of transgenic *Brassica napus* plants showing resistance to three separate herbicides, glyphosate (Roundup), a sulfonylurea, and glufosinate ammonium (Ignite or Basta).

Using genetic markers, it was determined in the late 1970s that the level of pollen contamination from commercial rapeseed fields onto 46–meter-square isolation plots located 46, 137, and 366 meters distant, was 2.1, 1.1, and 0.6%, respectively, for the *B. napus* species and 8.5, 5.8, and 3.7% for *B. campestris*. No border effects were detected, nor was the level of contamination affected by the direction in which the isolation blocks were oriented to the contaminant source. These and other data from cytoplasmic male sterile rows grown at increasing distances from a pollen source indicate that, although a very large proportion of the pollen cloud falls to the ground within a few meters, under favorable conditions a small proportion of contaminating pollen can move long distances by insect and/or perhaps wind transport. Thus we know that pollen from these species can be carried a considerable distance and perhaps larger quantities of *B. campestris* pollen are capable of moving farther than *B. napus*. The question remains, however, as to what happens when the pollen lands on a foreign stigma surface.

To determine the ease of interspecific crossing among spring forms of *B. napus, B. campestris (B. rapa), B. juncea, B. nigra* and *Sinapis arvensis (B. kaber),* these species were artificially crossed using bud pollination in the greenhouse. Several of these species were also co-cultivated in field plots and the progeny examined for the presence of natural hybrids.

Of greatest interest are interspecific crosses with the important weed, wild mustard *(S. arvensis).* When this species was reciprocally crossed with *B. campestris* and *B. napus,* no hybrid seeds were obtained, even though pollinations were made on emasculated buds in favorable greenhouse conditions. Similarly, no hybrids were obtained in the cross *S. arvensis* x *B. juncea,* although the reciprocal cross produced 2.5 hybrid seeds per 100 bud pollinations. F_1 plants were backcrossed to *B. juncea,* one non-viable seed was produced in 1.003 pollinations. Similarly, in 1881 backcrosses to *S. arvensis,* one BC_1F_1 seed was obtained, but the plant resulting

from this seed was completely sterile. Thus, gene transfer to *S. arvensis* from the three major oil crop species, *B. napus, B. campestris* and *B. juncea,* was not achieved even under the most favorable conditions, and no hybrids were identified from natural crossing of these species when they were co-cultivated in field plots over a three year period.

In crosses between *B. nigra* and *S. arvensis,* hybrid seeds were readily obtained when *B. nigra* was the female and to a lesser extent when the reciprocal cross was made. The homology between these two species strongly suggests that *B. kaber* would be a more appropriate species designation than *S. arvensis.*

When the amphidiploids *B. juncea* and to a lesser extent *B. napus* were used as females in crosses with diploid *B. nigra,* 3 and 0.9% of the artificial pollinations resulted in a hybrid seed, while the reciprocal crosses were successful in hybrid seed production only to the level of 0.5 and 0.1%. When F_1 plants of the *B. juncea* x *B. nigra* cross and its reciprocal were pollinated by *B. nigra* or self-pollinated, no seeds were produced. However, when these F_1 plants were pollinated by *B. juncea,* some seed was set. Thus, if this cross were to occur in either direction it is highly unlikely that a *B. nigra*-like plant would emerge. If any backcross plants were to survive, they would be of a reconstructed *B. juncea* genotype. Hybrids from reciprocal crosses of *B. napus* x *B. nigra* were highly sterile and no seed was obtained on selfing. A few seeds were produced on the F_1 interspecific hybrids when they were pollinated by *B. napus* pollen but no viable seed could be produced in backcrosses with *B. nigra.* The level of infertility of the interspecific F_1's when open-pollinated, selfed or backcrossed to *B. nigra* in these experiments clearly demonstrates the severe natural barrier to gene transfer from *B. napus* and *B. juncea* to *B. nigra*.

Under co-cultivation in the field, the only interspecific hybrids obtained were from the crosses *B. napus* x *B. juncea* and *B. napus* x *B. campestris.*

The data from this interspecific crossing experiment and evidence from the literature indicate that the opportunity for gene transfer directly from *B. napus* or *B. juncea* to *S. arvensis* is essentially zero. The possibility of gene transfer to *S. arvensis* from *B. napus* and *B. juncea* using *B. nigra* as a bridge, is also remote. This conclusion is based on the fact that we were not able to get selfed seed to set on the F_1 hybrids, nor were we able to obtain backcrosses to *B. nigra.* In addition, it is known that *B. nigra* is not a common weed of western Canada and rarely, if ever, is found in association with *B. napus, B. juncea* or *S. arvensis.* Even in eastern Canada, where *B. nigra* is found in the Great Lakes region, it does not have a wide distribution and is normally a weed of waste places rather than cultivated fields. On the other hand, the ease with which interspecific crosses were obtained both in the greenhouse and under field conditions between *B. napus, B. campestris* and *B. juncea* suggests that gene transfer among these species in western Canada could, and perhaps does, occur in nature.

The present Canadian regulations for small plot testing of Brassica oilseed transgenic materials appears to be more than adequate. However, there is an area of uncertainty as to the controls that may be imposed as we move from testing in an isolated transgenic test block to cooperative field trials or to field-scale multiplications for pilot plant extraction and product evaluation. From our experience, we would recommend that each introduced gene should have its own risk assessment and that if no hazard is identified within a three-year isolation test and evaluation program, the transgenic material should be handled in the same way as we would introduce a mutated strain into the testing and evaluation system. None of the transgenic brassicas that we know today appear to pose a threat to the environment since they can all be controlled with presently available herbicide and cultivation techniques.

Paper 4

Stephen Gleddie
Agriculture Canada, Plant Research Center, Canada

In order to rank the potential risks associated with field tests of transgenic crucifers containing the various types of genes which were mentioned on October 9, 1990, I have used the following rationale. The disease and insect resistance genes that were mentioned do not pose any risk at all in my opinion. I believe that these two objectives are components of many conventional breeding programs of oilseed crucifers. If we are concerned about gene flow of these traits from cultivated oilseed crucifers *(Brassica napus, B. campestris, B. juncea)* into cruciferous weeds then we must evaluate the objectives of any breeding program. Surely it is recognized that plant breeders have been actively searching for novel sources of disease and insect resistances for many years. And when these traits are found, they have been incorporated into the gene pool(s) of the crop plants. Can anyone find any example of such a trait which has 'escaped' from the crop and caused the weed population to become a more adapted pest? Surely the models are in place to test this since oilseed crucifer breeding programs have been established for many years (30 to 40 years).

When the discussion of altered protein, oil and carbohydrate metabolism was held, I sensed that certain panelists were expressing the same concerns mentioned above—namely that these traits, should they 'escape,' would cause increased fitness in the weed population. However, as rapeseed breeding programs established 30–40 years ago in Canada have shown, it is possible to make massive strides by "conventional plant breeding." This has resulted in much higher oil and protein content, in quality modifications (canola) of specialty oil cultivars, etc., etc. Yet after all of this effort to "improve" the crop, I am not aware of any measured changes or alterations to wild crucifers and weeds. In my opinion, the debate over the consequences of gene escape was a moot debate when it concerned the escape of disease resistance, insect resistance and quality traits.

When the debate over the consequences of gene escape switches to herbicide resistance, I believe that this is not a moot

debate. It is possible under these conditions that escaped genes would survive in the wild due to selection pressure. However, with proper crop rotations, this should not be a problem. As was pointed out from the floor (Dr. Pierce, Dupont) the potential for herbicide resistance/tolerance transfer from the crop species into weedy species has existed in the past. When herbicides are found to be selective against specific weeds (wild mustard) while the crop plants are naturally resistant *(B. napus, B. campestris)*, then a perfect model is established to test the potential movement of resistance gene(s) from the crop into the weeds. One such model system comes to mind. The Dupont herbicide "Muster" is registered in Canada to control wild mustard in canola since *B. napus* and *B. campestris* are naturally tolerant. Although these are not transgenic brassicas, it should be possible to test the movement of resistance gene(s) from canola into wild mustard under field conditions of various herbicide regimes. This simple assessment may provide some data about the rate of spread of herbicide resistance under various levels of herbicide application, and various rotations of herbicides.

Because I do not view the increase in weediness as a serious problem in transgenic oilseed crucifers, I will not address this issue.

In conclusion, I found some of the arguments regarding the consequences of gene escape to be rather obscure and hypothetical, while our knowledge and past experience with plant breeding and agronomy was often forgotten or overlooked.

The third major question dealt with safeguards and I noted the plea or point made by Paul Williams that adequate funding be maintained for the various gene banks and plant genetic resources units. This plea was for continued support for proper isolation facilities and good pollination control systems (tents). If the proper care and handling of important genetic resources is practiced then I think we have done what is necessary to maintain the genetic integrity of these resources.

It is certainly my opinion that transgenics will one day become a routine part of agriculture. We need to address the questions and points that were raised at this meeting so we are confident about safety. As you can gather from my opinions, public and environmental safety is not compromised by field tests of transgenic oilseed crucifers providing the appropriate safety measures are taken.

Paper 5

Rebecca J. Goldburg
Environmental Defense Fund, New York

Introduction: Focus of My Comments

I will limit my comments in two ways. First, transfer of genetically engineered traits from transgenic crop plants to their related plants is widely considered to be the major environmental risk of introductions of transgenic crop plants (e.g. Center for Science Information, 1987; Hoffman, 1990). My comments focus on (a) the likelihood of gene transfer from transgenic oilseed crucifers to other crucifers, (b) the environmental consequences of gene transfer, and (c) an example of the sort of natural history information and data from experiments that will be needed in order to make informed decisions about the consequences of gene transfer.

Second, although oilseed crucifers are not now particularly; common crops in the United States, a number of crucifer species have been grown for oil in various regions of the world. These include *Brassica campestris, B. nigra, B. napus, B. juncea, Raphanus sativus, Sinapis alba, Eruca sativa, Crambe maritima, C. abyssinica,* and *Camelina sativa* (Crisp, 1976). I will limit my comments to the three oilseed crucifer species that are most actively being promoted as new crops to farmers in the United States: *B. campestris, B. napus,* and *C. abyssinica* (Erickson and Bassin, 1990). Varieties of *B. napus* and *B. campestris* that are low in glucosinolates and have an erucic acid content of less than 2% are known as canola (Cooperative Extension Service, 1990). Canola oil is consumed by humans, and defatted canola meal is consumed by livestock. High erucic acid varieties of *B. napus* and *C. abyssinica* can be grown to produce industrial oils. After detoxification, defatted industrial oilseed meal, like canola meal, can be consumed by livestock (Erickson and Bassin, 1990).

Likelihood of Gene Transfer

Brassica spp.:

Gene transfer from transgenic oilseed *Brassica* spp. to other crucifers could easily occur. *B. napus* and *B. campestris* can hybridize with a number of crucifer species. In trial crosses, for example, Yarnell (1956) found that *B. napus* hybridizes with *B. campestris* (including wild varieties and vegetables such as Bok Choi, which Yarnell classifies as separate species but are now considered as *B. campestris* [T. Mitchell-Olds, pers. comm.]) and *B. juncea* (brown mustard), although the fertility of the hybrids varies. Crosses with the weed *B. kaber* (now commonly called *Sinapis arvensis)* and the radish *R. sativus* are difficult, but possible. According to Kemp (1989), *B. napus* also crosses with the wild and often weedy plants *B. nigra* and *B. hirta.* Cultivated transgenic *B. napus* would also be expected to cross with weedy strains of *B. napus.*

Wild *B. campestris* crosses with cultivated *B. campestris* vegetables, *R. sativus, B. oleracea* (cabbage and other cole crops), and *B. carinata* (Abyssinian mustard) (Yarnell, 1956).

Most of the results discussed above are from artificial experiments, but cross-pollination between oilseed *Brassica* spp. and other crucifers could occur naturally in the United States. In Kansas, for example, oilseed crucifers bloom from late April through May. Weedy *B. campestris, B. juncea,* and *B. kaber* also bloom in Kansas at this time (Gates, 1941) and thus are available for cross-pollination. Moreover, both *B. campestris* and *B. napus* are insect pollinated, with honey bees acting as important pollinators (T. Shistar, pers. comm.). Since honey bees tend to range as far as two to three miles from their hives (Kansas Agricultural Experiment Station, 1977), cross-pollination could occur between *Brassica* spp. a good distance apart.

Cross-pollination between cultivated high erucic acid rapeseed and low erucic acid canola is already a problem. It results in oilseeds with intermediate levels of erucic acid that are unfit for human consumption and of low value for industrial oils (Van Dyne et al., 1990). To avoid such problems from cross-pollination, Idaho established six rapeseed production areas in 1986.

Crambe abyssinica:

Transgenic *C. abyssinica* may be less likely than transgenic *B. napus* or *B. campestris* to transfer genes to other crucifers. According to Van Dyne et al. (1990), *C. abyssinica* does not cross-pollinate with canola or industrial rapeseed (although no reference is given for this assertion).

Conclusion:

Gene transfer from transgenic oilseed *Brassica* species to other crucifers could readily occur—given that transgenic crucifers have flowering phenologies and spatial distributions that overlap with other crucifers. It may be possible in some small-scale experiments to genetically isolate transgenic crucifers from other crucifers, for example by ending the experiment before anthesis. However, because theoretically only one gene transfer event is needed to introduce a gene to a population, and use of commercially available seeds is difficult to closely control, it will be virtually impossible to genetically isolate commercially available transgenic oilseed crucifers.

Environmental Consequences of Gene Transfer

The environmental consequences of gene transfer from transgenic oilseed crucifers to other crucifers will depend, in part, on whether the recipient crucifer is a crop or wild plant. Because industrial oilseed crucifers are not intended for human consumption, genes encoding industrially valuable traits, or pesticidal compounds that are hazardous to humans, could someday be genetically engineered into oilseed crops. This could result in problems analogous to those that already exist from cross-pollination between low and high erucic acid oilseeds, as discussed above. Cross-pollination could hypothetically lead to engineered toxins being present in canola seeds, or possibly even mustard seeds intended for spices or condiments.

Gene transfer from transgenic oilseed crucifers to wild crucifers could lead to undesirable consequences in some instances. Transferred traits, such as drought or salt tolerance, or resistance to insects or pathogens, could confer an advantage to wild crucifers and spread via natural selection. Armed with their unique genetic advantage, plants with such acquired traits could displace populations of the same and other species (Wilson, 1990). Thus, erosion of genetic diversity, already a considerable problem in plants, could be exacerbated. A single instance of such genetic erosion, without other immediate obvious consequences, might in some people's eyes not be a serious loss. Nevertheless, the cumulative effect from wide uncontrolled use of transgenic plants could be considerable.

Because most, if not all, wild crucifers that cultivated oilseed crucifers are known to cross-pollinate are not native to North America, displacement of populations of native wild plants by wild crucifers could be a "double whammy" to conservation. The population size or geographic distribution of an exotic species would not only increase, but the increase would occur at the expense of one or more native plant species. On the other hand, any genetic erosion that occurred within a population of wild non-native crucifers as a result of gene transfer would likely not be of concern to conservationists.

In at least three ways, transfer of a single gene to a wild plant could have severe consequences beyond genetic erosion. First, a transferred gene could confer a significant advantage to a wild plant and increase problems it causes as a weed. Gene transfer from transgenic oilseed crucifers could have such a result, particularly because *B. campestris, B. nigra, B. napus, B. hirta, B. juncea,* and *R. sativus* are all considered weeds, and *B. kaber* is considered a serious weed (Agricultural Research Service, 1971; Office of Agricultural Biotechnology, 1990).

Second, transfer of a pest-resistance gene could cause populations of a wild plant to no longer be available to animals, such as butterflies, that depend on it for food. This could lead to a decline in populations of the affected animal. This problem is, however, unlikely to arise from gene transfer by oilseed crucifers in North America. Most, if not all, wild crucifers that cultivated oilseed crucifers are known to cross-pollinate are non-native. Thus, it is unlikely that native animal species specialize on these wild crucifers for food.

Third, transfer and spread of a gene-conferring herbicide tolerance to a weedy brassica could lessen the usefulness to farmers of the herbicide to which tolerance was conferred. This could have undesirable environmental effects if, in order to control the tolerant weed, farmers applied larger quantities of herbicide or especially environmentally "harsh" herbicides. (Of course, herbicide-tolerant oilseed crops could themselves have similar undesirable environmental effects, by changing patterns of herbicide use [see Goldburg et al., 1990].)

Evaluating the Consequences of Gene Transfer

Evaluating the consequences of gene transfer from a particular transgenic oilseed crucifer to a wild crucifer may often require ecological studies. Although transfer of certain traits, such as pest resistance and stress tolerance, has the potential to confer a selective advantage to wild crucifers, deciding whether the trait actually will confer a selective advantage may require a modest to considerable amount of field research.

Consider the example of a "B.t." gene conferring resistance to lepidopterans. Transfer of a *Bacillus thuringiensis* (B.t.) gene could confer a selective advantage to a wild plant if the plant's reproductive success is limited by phytophagous lepidopterans. Steps to evaluate this possibility might include the following:

- Survey scientific literature and natural populations of the wild crucifer to establish which lepidopterans feed on it. Because insect host plant choices and population levels can vary considerably in space and time (Fox and Morrow, 1981), such surveys should not be restricted to just a few articles, places, or days.

- Given that lepidopteran "X" does feed on the wild crucifer, check to see if it is on state or federal lists of threatened or endangered species.
- Since not all lepidopterans are equally susceptible to B.t. delta endotoxins, feed lepidopteran "X" tissue from transgenic plants to evaluate the insect's susceptibility.
- Perform field experiments to see if lepidopteran "X" affects the reproductive success of the wild plant. This could be accomplished through experiments in which the lepidopteran was excluded (e.g. by cages or "Tanglefoot") from some wild plants but not others. Various indicators of plant health as well as direct indicators of reproductive success, such as seed set, could be measured as responses to the exclusion experiments. (See Louda [1984] for an example of an experiment demonstrating that an insect herbivore significantly reduced the size and fruit conditions that exist in natural environments, such experiments would likely need to be performed in more than one field season and location.)

Acknowledgment: These comments were prepared with the assistance of Terry Shistar.

References:

Agricultural Research Service. 1971. Common Weeds of the United States. Dover, New York, NY.

Cooperative Extension Service, 1990. Canola Production Handbook. Kansas State University, Manhattan, KS.

Crisp, P. 1976. Trends in the breeding and cultivation of cruciferous crops. In: The Biology and Chemistry of the *Cruciferae,* J.C. Vaughan, A.J. Macleod, and B.M.G. Jones, (eds.). Academic Press, New York.

Center for Science Information. 1987. Regulatory Considerations: Genetically Engineered Plants. Summary of a workshop held at the Boyce Thompson Institute for Plant Research at Cornell University. San Francisco, CA.

Erickson, D.B., and P. Bassin. 1990. Rapeseed and Crambe: Alternative crops with potential industrial uses. Agricultural Experiment Station, Kansas State University, Manhattan, KS, Bulletin 656.

Fox, L.R., and P.A. Morrow. 1981. Specialization: Species property or local phenomenon. Science 211:887–893.

Gates, F. 1941. Weeds of Kansas. Kansas State Board of Agriculture, Topeka.

Goldburg, R., Rissler, J., Shands, H., and C. Hassebrook. 1990. Biotechnology's Bitter Harvest: Herbicide-Tolerant Crops and the Threat to Sustainable Agriculture. The Biotechnology Working Group.

Hoffman, C.A. 1990. Ecological risks of genetic engineering of crop plants. BioScience 40:434–437.

Kansas State Agricultural Experiment Station. 1977. Bee Culture. Kansas State University, Manhattan, KS.

Kemp, J. 1989. Draft example of classifying unmodified organisms. Agricultural Biotechnology Research Advisory Committee of USDA.

Louda, S.M. 1984. Herbivore effect on stature, fruiting, and leaf dynamics of a native crucifer. Ecology 65:1379–1386.

Office of Agricultural Biotechnology. September 11, 1990. Draft Guidelines for Assessing the Environmental Impact of Research Involving Planned Introduction into the Environment of Organisms with Deliberately Modified Hereditary Traits. USDA, Washington.

Van Dyne, D.L., Blase, M.G., and K.D. Carlson. Industrial feedstocks and products from high erucic acid oil: Crambe and industrial rapeseed. University of Missouri, Columbia.

Wilson, H.D. 1990. Gene flow in squash species. BioScience 40:449–455.

Yarnell, S.H. 1956. Cytogenetics of the vegetable crops. II. Crucifers. The Botanical Review 22:81–166.

Paper 6. Some Considerations on Introductions for Field Research and Commercial Production

Ralph W. F. Hardy
Boyce Thompson Institute for Plant Research at Cornell, Inc., New York

My comments are based upon participation, during the last several years, in several groups that have identified principles for introduction of transgenic plants for field research and upon my developing thoughts on principles to guide large-scale commercial production. The principles for decision-making for field research will be summarized, and their relevance to transgenic crucifers and to other transgenic plants is stated.

> A recommendation is made to perform field research with easily measured, environmentally neutral marker genes in transgenic commercial crucifers to quantitate gene flow, if any, to wild and weedy relatives such as mustard and stink weed that are found in areas of commercial production of crucifers. It is also recommended that maps with ecological profiles of the relatives of crucifers be made with the data entered into the public database.

Risks of field introduction for research on transgenic crucifers is expected to be extremely low based on the considerable relevant experience with traditional plant breeding of domesticated crops including crucifers. The risks for large-scale commercial production may, on occasion, be larger than for field research based on the commercial production experience of traditional crop agriculture. Significant risks have occurred only on rare occasions: these occasions, in general, accompanied the major adoption of a crop genotype, e.g. male-sterile cytoplasm corn or an agrichemical, e.g. systemic fungicide where massive and continuous use led to a weakness, e.g. southern corn blight and benomyl pesticide resistance.

> It is recommended that a process be established to minimize the risks of commercial production of transgenic crops including crucifers.

Field Research

The published reports of several committees have identified principles for decision-making on field introduction of transgenic plants (Boyce Thompson Institute, 1988; Brookings Institution, 1987; General Accounting Office, 1988; National Academy of Sciences (NAS), 1987; NAS 1989; Office of Technology Assessment, 1988; and Tiedje, et al, 1989). The 1987 National Academy of Sciences (NAS) report, *Introduction of Recombinant DNA-Engineered Organisms into the Environment: Key Issues* (NAS, 1987), concluded that risk is associated with a genetic product rather than the process—e.g. traditional plant breeding, cellular techniques, or molecular techniques—used to produce that product. In late 1987 a highly focussed workshop at Boyce Thompson Institute on *Regulatory Considerations: Genetically Engineered Plants*[3] led ecological and genetic scientists to conclude that there is negligible agricultural or environmental risk in the near term for most field releases for research and commercial use of genetically engineered major crops. Crucifers were not considered since they are not one of the top fifteen major U.S. crops. It was recommended that maps should be made and ecological profiles completed of the relatives of crop plants, and these data should be entered into a public database. At this crucifer workshop, it is recommended that such maps be produced for the world areas where field tests and commercial productions of crucifers occur or are projected. In 1989, the National Research Council published *Field Testing Genetically Modified Organisms: Framework for Decisions* (NAS). This report documented the relevant and remarkably favorable experience base of safe field introductions for research purposes of hundreds of millions of novel genotypes of crop plants produced by plant breeding. Experience with exotic plants was concluded to be not relevant to transgenic crop plants. Weediness is the major concern identified for transgenic plants, but it was noted that gene transfer from domesticated crop plants to wild and weedy relatives where such relatives exist would domesticate the weedy relatives and thereby decrease their competitiveness. A three-step decison tree identified familiarity/experience, containment, and risk as the key considerations with examination in the above sequence. This decision tree can be applied to genetically modified crops produced by traditional plant breeding as well as by cellular or molecular techniques. The decision tree should apply to transgenic crucifer crops as to other transgenic crops. In addition, it was noted that the molecular techniques allow appropriate risk questions to be asked and answered with a greater precision than for traditional or cellular techniques.

It is recommended that information on gene flow for crop crucifers be collected under field conditions in different geographic areas if it has not already been done or is in process. Transgenic crucifers with an easily measured and environmentally neutral marker gene, e.g. bacterial or firefly luciferae, could be used. Movement into native plants such as stink weed or mustard may be of interest in Canada and the United States.

Commercial Production

Based on agricultural experience, the risk from large-scale commercial production of domesticated crops may, on rare occasions, be greater than the risk of introductions for field research. For example, the large-scale use of a specific male-sterile cytoplasm genotype with advantages for hybrid corn production led to a major problem of susceptibility to southern corn blight and a major corn-yield loss in a single year. The male-sterile cytoplasm genotype was replaced in succeeding years to eliminate the blight problem. A similar example for agrochemicals is the development of pesticide resistance National Research Council, 1986). The dominant and continuing use of an advantageous systemic fungicide *Benlate* in the 1970s led to the development of resistance to the fungicide. Crop agriculture risks, when they have occurred with novel genotypes or agrochemicals, have arisen from dominant and continuous use of the advantageous genotype or agrochemical. Similar risks may, on occasion, occur with transgenic crops such as transgenic crucifers. It is recommended that systems be established to record areas where specific transgenic crops are grown. Where problems may develop such as pesticide-resistant insect pests, consideration should be given to limit the continuous growth of the insect-resistant transgenic crop.

Summary

Principles for introduction for field research of transgenic crops are applicable to crucifers. Maps should be made of the ecological profiles of native relatives of crop crucifers. Minimizing the rare but real risks of dominant and continuous use of a specific genotype may be the major risk for commercial production of any transgenic crop including transgenic crop crucifers.

References:

Boyce Thompson Institute. 1988. Regulatory Considerations: Genetically Engineered Plants. Center for Science Information, San Francisco.

Brookings Institution. 1987. Public Policy and the Release of Genetically Engineered Organisms into the Environment. The Brookings Institution, Washington.

General Accounting Office. 1988. Biotechnology: Managing the Risks of Field Testing Genetically Engineered Organisms. GAO-RCED-88-27. Washington.

National Academy of Sciences. 1987. Introduction of Recombinant DNA-Engineered Organisms into the Environment: Key Issues. National Academy Press, Washington.

National Academy of Sciences. 1989. Field Testing Genetically Modified Organisms: Framework for Decisions. National Academy Press, Washington.

National Research Council. 1986. Pesticide Resistance: Strategies and Tactics for Management. National Academy Press, Washington.

Office of Technology Assessment. 1988. New Developments in Biotechnology—Field Testing Engineered Organisms: Genetic and Ecological Issues. OTA-BA-350, U.S. Govt. Printing Office, Washington.

Purchase and MacKenzie. 1990. Agricultural Biotechnology: Introduction to Field Testing. USDA—Office of Agricultural Biotechnology, Washington.

Tiedje, J. M., Colwell, R. K. Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., and P. J. Regal. 1989. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. Ecology 70: 298–315.

Paper 7
Environmental Consequences of Gene Escape: Weeds

Kathleen H. Keeler
Department of Biological Science, University of Nebraska

Risk

In the study of risk assessment the risk is considered the product of the hazard times the exposure (r=h*e). If there is no hazard, or no exposure, there can be no risk. A similar approach to assessing the potential problems from genetically engineered (transgenic) organisms in the environment sets the risk of a problem from transgenic organisms or transgenes as equal to the probability that the transgenic organisms or genes will cause a problem (hazard) and the probability that the organisms or genes will escape from the intended location(s) to sites where they can cause a problem (exposure). This approach seems appropriate for release of transgenic organisms since 1) it draws on an established methodology, 2) is quantitative or potentially so, and 3) assumes that a very complex problem can be broken into independent and manageable component parts for analysis.

The most likely and potentially serious risk of releasing transgenic plants is the generation of new, serious weeds (e.g. Colwell et al., 1985; Hauptli et al., 1985; Tiedje et al., 1989). This seems particularly likely for brassica, given the array of weeds already present in the family and genus (e.g. Holm et al., 1977; Rollins, 1981; Beversdorf, 1987). I confine myself to the problem of weeds produced by transgenic plants here. My definition of weed is "a plant that interferes with human activity" (Salisbury, 1961; Buchholtz, 1967) and the focus is on agricultural weeds and plants that might move from croplands into surrounding habitats.

Analysis using the risk assessment model proceeds as follows:

There is no risk if there is either no probability of a negative effect or of escape. Let me take these in reverse order.

Exposure: Escape

I will assume that transgenes and transgenic plants in the field where they were planted are not a problem. Agricultural research should produce crops that are under control within the fields where they were planted. Exposure in risk assessment thus means: Will the transgenes or transgenic plants leave the field to cause problems? The possible methods for leaving the field to cause a problem are 1) production of populations of the crop that act as weeds, and 2) gene exchange (hybridization) with weedy wild relatives so that the weed populations possess and benefit from the transgene. I am disregarding the possibility that plant genes are transferred other than via pollination, because I know of no cases of natural horizontal transfer between higher plants.

1) Populations of the crop as weeds. The data is not yet all in as to whether our modern crops can readily act as weeds, or whether weediness requires substantial allele substitution from the crop plant phenotype (Keeler, 1989; Fitter et al., 1990; Keeler, 1990). *Brassica* provides a group in which the seriousness of crop varieties acting directly as weeds can be evaluated. If crops were to act as weeds without further evolution, the first place this would be expressed is in carryover. Crops frequently germinate or resprout in the field the following year, in the presence of a new crop. While methods for controlling carryover weeds seem to be generally effective, brassicas do present a serious weed problem due to carryover and any transgene that might enhance that weediness needs to be taken very seriously.

While any transgenic crop that poses a problem as a carryover weed is going to be a weed problem, transgenic crops that do not cause immediate problems may form ruderal populations that are problematic later, or invade surrounding habitats. Transgenic crops that are invasive need to be prevented but will be harder than carryover weeds to assess, monitor, and eliminate.

2) Hybridization with weedy wild relatives. In this case, the transgene moves to a genome that already has an effective weed phenotype. If the transgenes give this weed enhanced success as a weed, this could be serious indeed.

However, the mere presence of a wild relative growing around the field does not signal that transgenic brassicas should be avoided. Not all relatives, including members of the same genus, hybridize with a crop and many fewer will hybridize under field conditions. Thus, the crop and the relative must cross under the specific growing conditions for the genes to escape. However, plants which cross "rarely" represent a situation in which caution should be exercised: in the case of hybridization the improbable event can have too serious consequences to be ignored because of a low probability (e.g. Colwell, 1988). For *Brassica* there are excellent data on these relationships.

There is an additional criterion for evaluation of crossing with wild relatives: are the relatives weeds? There are many plants that are very abundant without being treated as weed problems. This is another situation in which it would be extremely costly to be wrong, but a transgene that escapes into

a common wild relative of the crop does not pose a problem unless that relative interferes with human activity. If, with or without the transgene, the relative is abundant but not weedy, there is no weed problem. I have in mind *Raphanus raphanistrum*, the wild radish, in northern California. It is very abundant but is not listed among California's noxious weeds (California Dept. of Agriculture, 1988). Again, the dangers of being wrong in the assessment here should be weighed carefully, because if a plant that is very abundant becomes noxious there is an immediate and serious weed problem.

Practical solutions to the problem of hybridization with wild relatives will require detailed information on the distribution of the wild relatives and on which ones are compatible with the crops, something that could readily be generated for *Brassica.*

Differences between the two types of weed production include: i) carryover can occur anywhere *Brassica* is grown, while hybridization can occur only where the wild relatives exist, although wild relatives have been widely introduced (e.g. Holm et al., 1979; Rollins, 1981), and ii) methods to control carryover can be worked out in advance by seriously considering how to control transgenic brassicas should they carryover. With sufficient vigilance, naturalizing populations of the crop can be prevented as well. In contrast, the wild brassicas that are considered weedy receive that designation because they are *already* difficult to control. With a transgene, they can be expected to get worse. Thus, weedy populations of the crops are potential problems everywhere the crop is planted but controllable, while weed problems with hybrids will be confined to specific areas (where weedy compatible relatives are found) but are more likely to be extremely serious.

For *Brassica,* both the crop as a weed and hybridization with weedy wild relatives need to be taken seriously. However, to safely manage transgenic plants, if exposure can be reduced toward zero, for example by releasing plants which can be efficiently and cheaply eliminated to prevent carryover and naturalization where there are no compatible weedy relatives present, then the risk approaches zero, whatever the nature of the transgenes.

Hazard: New Weeds

If the exposure is not zero—the current situation—then the nature of the transgenes must be considered. If those pose no hazard, there is no risk, whether or not there is exposure.

Nontransgenic brassicas are assumed to pose no hazard, since they are currently widely grown crops. Thus, transgenic brassicas will only become a hazard if the transgenes change their phenotype (the way genes are expressed in the whole organism), or escape to change the phenotype of some other organism.

The first step, which seems straight-forward, is to determine whether the particular transgene can change the crop or hybrid brassica in such a way as to create a worse weed. If the answer is no, then there is no hazard and so no risk of a problem.

Actually, this analysis is not as easy as it appears at first glance. I wrote that the criterion is the creation of a "worse weed" but in fact this point needs discussion. Some brassicas are economically important weeds. If a new variety with the same properties, and no worse, than one of the existing weeds is produced, is this a hazard or not? The easy response of "no hazard" may depend on the situation. Consider this example: weedy brassicas with naturally occurring resistance to the herbicide triazine exist (LeBaron, 1984; Beversdorf, 1987). A crop with those same exact herbicide resistances, growing in the area where the naturally occurring resistant brassicas are found, will pose no additional problem to agriculture and so no hazard. However, in areas where weedy brassicas occur but not triazine-resistant brassicas, or where triazine resistance is found but not in the weedy brassicas, the appearance of triazine-resistant weedy brassicas does pose a hazard to agriculture. To release triazine-resistant brassicas where the weeds are triazine resistant would not pose a risk (but is pointless), but triazine-resistance genes do have the potential to produce a worse weed where the genes are not yet found. A practical solution to this problem is not obvious to me.

What is obvious, however, is that genes which offer no prospect of enhanced weediness can be released whether or not they escape. Oil quality, date of maturity, seed presentation—a number of genes may fall into this category. It is impossible to give a blanket exemption for any trait, however, because somewhere there is an environment in which this gene raises the fitness of a weed. If the trait that changes the oil quality of the seed made the seeds attractive to seed-gathering ants, they might carry them away, bury them in their nests, and in so doing turn the crop into an invader of surrounding deserts. Nevertheless, many genes can be agreed to be innocuous in real-world environments, especially after a few experiments on the performance of the transgenic phenotype.

In addition, some genes will alter the phenotype without expanding the range of variation of the species: if the transgene changes a phenotypic characteristic of a cultivar in which the crop already has a great deal of natural variation, it is possible to compare the transgenic phenotype to previously existing phenotypes, and determine that the tolerances of the transgenic plant fall within the range and so present no increased hazard. For example, an agronomically useful trait which confers, in addition, some cold-tolerance to the variety but does not extend that variety's cold tolerance outside the range of cold tolerance for other *Brassica* varieties can be effectively compared to the record of the most cold tolerant variety in terms of weed potential. If it is less tolerant than other varieties and they are not weed problems, this change of phenotype due to the transgene can be concluded not to enhance weediness.

For an array of traits it should be possible to either establish that each trait does not enhance weediness in any abundant environment and is theoretically unlikely to do so even in impossible circumstances. Other traits can be shown to fall within the existing range of variation of the crop so that the trait will not expand weed potential. Both sets of exemptions have potential for failure, and the consequences of making an error with a particular trait need to be taken into consideration. Note the point made above that just because the same trait occurs somewhere in the species does not mean that the trait may not pose a hazard. Nevertheless, it seems probable that an array of innocuous transgenes exist and, if hazard is zero, risk is zero.

This brings us to the not-necessarily-innocuous transgenes. For these, the potential for negative effects from the transgenes must be analyzed and a set of criteria about tolerable probabilities of problems established. Potential risk vs potential benefit analyses seem more useful than analyses based on risk alone, because, as I see it, that better reflects actual human decision-making.

The releases that will cause the most debate will probably either offer great benefit to agriculture but have relatively high potential for producing weeds, or involve a transgene that is perceived as benign by some groups and highly hazardous by others. I would like to make a few comments about the approaches for evaluation of such releases.

1. Not all conflicts are resolvable to everyone's satisfaction, because not everyone will accept compromise solutions. However, some of the differences in perception come from different assumptions and goals and making those explicit will help.

For example: where is the transgene likely to be a weed? Agriculturalists are more concerned about weeds of agriculture: environmentalists are more concerned about weeds of natural areas. Thus, a minor crop weed with potential to invade native meadows at the expense of small native crucifers would be strenuously objected to by the environmentalists although agricultural extension weed specialists, if polled, might conclude this plant was too unimportant to require control efforts.

Likewise, what traits are high hazard? Herbicide tolerance, as an escaped trait, raises anxiety in the hearts of any company trying to develop viable crop varieties, since the herbicide-resistant wild relative will eliminate the market for the resistant crop. Range and natural area managers give little attention to the production of herbicide-resistant races because range managers cannot afford herbicide as a weed control option and natural areas contain too many susceptible species for herbicides to be used in weed control. Thus, the selective advantage that herbicides confer is missing from rangeland and preserves, and so such races offer little threat.

2. Ecology is changing like other fields. As in all fields, the textbooks lag. I want to draw attention to a principle of ecology that has worrisome implications for the release of transgenic crops.

Many of the genes that can effectively be transferred to plants and that show economic potential are genes which confer biotic resistance, that is, resistance to other organisms including herbivores or diseases. Even herbicide tolerance is a biotic resistance, in the sense that it allows application of herbicides to prevent competition from other plants (weeds) from reducing the crop yield. Generally, the literature indicates that plants are limited by abiotic environmental factors, such as rainfall temperature and salinity, so it is not immediately obvious that the success of biotic resistance genes will enhance weediness. However, while ecologists are currently trying to determine the relative importance of biotic interactions in limiting plant numbers, there is agreement that biotic interactions *can* and sometimes do limit plant numbers. Thus: i) biotic interactions can limit plant abundances, i.e., keep plants under control, and ii) a weed is a plant whose numbers are out of control, or, which is more abundant than people like (the same plant if infrequent is not a weed problem). The conclusion is that changes in biotic interactions can cause weed problems. If biotic interactions—competitors, disease, herbivores—keep a plant relatively rare, it is not a weed problem. Should it become resistant to these the biotic interactions, more seeds will be set, and the numbers might rise to a level that it becomes a problem weed. All transgenes that alter biotic interactions have the potential for increasing the abundance of the plant and therefore increasing its weediness, as a consequence of these very basic principles of population regulation.

What does this mean for brassicas? It means that transgenes for herbicide resistance, insect resistance and disease resistance all have the potential for causing a weed problem. The way herbicide resistance can raise weed fitness has received a lot of attention, so I'll consider insect and disease resistance. If weeds lose substantial fitness to insect damage or disease then the current status of the weed requires losses to those enemies. Protection will raise their fitness, and therefore change their weediness, and a more fit weed is a more serious weed.

Basic interactions *can* limit plant abundance: but how important is it really? There is little direct information. However, let me suggest that the field trials to see if the transgene is worth pursuing are a good set of preliminary studies on the value of the gene to the weeds. A transgene that is valuable in crop protection demonstrably lowers damage to the plant and raises production. For crops like oilseed rape, effective protection means more seed production. I contend that this is directly relevant data on how a weed with the transgene would fare. The weed is either a naturalizing population of the crop itself or a weed so similar to the crop it will cross with it. This suggests that almost always, the crop and the weed will be sufficiently similar that pests of one are pests of the other. Therefore, if pest resistance can allow greater seed production by the crop, there is no reason to expect it not to allow greater seed production by the weed. Weeds that produce more seeds are going to be more abundant.

The principle that biotic interactions that are changed by transgenes could be the ones controlling weed populations is one of the reasons for ecological concern about releases. A weed with enhanced fitness could invade natural areas. Like the situation with herbicide-tolerance described above, however, escape of a successful transgene will affect growers first. The lack of concern about such escape by breeders suggests that detailed knowledge about the brassica weeds indicates they are not limited by biotic interactions. If this were documented, it could allay the concerns of many ecologists and environmentalists.

One final point: there will always be the possibility of subsequent evolution of the plants. For example, a small naturalized population might become an enhanced weed due to a new mutation with synergistic effect on the transgene. This sort of problem is beyond the ability of regulators and breeders to prevent. If public interest groups thought that a transgenic weedy brassica even might, after attaining unexpected mutations, turn into a weed of the scale of water hyacinth, leafy spurge, or bindweed, they would be irresponsible not to recommend that the plant simply not be released: potential

costs outweigh potential benefits. How are we to get the benefits of genetic engineering for potentially high-risk situations? Immunology, needing antibody production but not wanting to introduce live viruses, developed attenuated viruses. The plant equivalent might be a male-sterile or apomictic cultivar. Biological containment of the transgene is the simplest safety device. In addition, approaching a high-risk release might be prudent to add the "Achilles' heel," additional control mechanisms (preferably a good one and a backup method) for the released plant. Some very clever ideas have already been suggested such as: specific pests that could be released later, nutritional requirements, and herbicide sensitivities. The extra control may well be worth the effort: research time may end up cheaper than the cost of generating adequate documentation on safety for regulators or winning in the courts against litigation to prevent release.

In sum, a risk assessment approach will allow analysis of the potential for environmental problems, and a variety of cultivars may either show no hazard or no exposure and so no risk. However, a variety of the most useful transgenes have the potential for considerable hazard. Finding cost-effective ways to reduce these hazards or eliminate the exposure part of the risk for these transgenes is the next challenge.

References:

Beversdorf, W. D. 1987. In: Biotechnology in Agricultural Chemistry. H. M. LeBaron, R. O. Mumma, R. C. Honeycutt and J. H. Duesing (eds.). Amer. Chem. Soc., Washington.

Boyce Thompson Institute. 1988. Regulatory Considerations: Genetically Engineered Plants. Center for Science Information, San Francisco.

Buchholtz, K. P. 1967. Weed 15: 388–389.

California Department of Agriculture. 1988. Noxious weed list. State of California, Sacramento.

Colwell, R. K. 1988. In: Safety assurance for Environmental Introduction of Genetically Engineered Organisms. J. Fiksel and V. T. Covello (eds.). Springer-Verlag, Berlin.

Colwell, R. K., Norse, E. A., Pimentel, D., Sharples, F. E., and D. Simberloff. 1985. Science 224:111–112.

Fitter, A., Perrins, J., and M. Williamson. 1990. Bio/technology 8:473.

Hauptli, H., Newell, N., and R. M. Goodman. 1985. Bio/technology, 33:437–442.

Holm, L. G., Pancho, J. V., Herberger, J. P., and D. L. Plunknett. 1979. A geographic atlas of the world's worst weeds. John Wiley and Sons, New York.

Holm, L. G., Plunknett, D. L., J. V. Pancho, and J. P. Herberger. 1977. The world's worst weeds. University of Hawaii Press, Honolulu.

Keeler, K. H. 1989. Bio/technology 7:1134–1139.

Keeler, K. H. 1990. Bio/technology 8:473–474.

Keeler, K. H., and C. E. Turner. 1990. In: Risk Assessment in Genetic Engineering: Environmental Release of Organisms. M. Levin and H. Strauss (eds.). McGraw Hill, New York.

LeBaron, H. M. 1984. Weeds Today. 15:2–6.

Office of Technology Assessment. 1988. New Developments in Biotechnology—Field Testing Engineered Organisms: Genetic and Environmental Issues. OTS-AS-350. U.S. Government Printing Office, Washington.

Rollins, R. C. 1981. J. Arnold Arboretum 62:517–540.

Salisbury, E. 1961. Weeds and Aliens. Collins, London.

Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., and P. J. Regal. 1989. Ecology. 70:298–315.

Paper 8
Risk Assessment of the Escape of Recombinant Genes from *Brassica*

Robin S. Manasse and Peter Kareiva
Department of Zoology, University of Washington

In the face of imminent field releases of transgenic plants, several environmentalists and scientists have expressed the general concern that organisms modified with recombinant DNA technology pose environmental risks that are distinguishable from those posed by plants that have been modified with traditional breeding methods. Some of these risks have been associated with the escape of recombinant genes from transgenic plants, through pollen movement and subsequent hybridization and selection, into populations of unmanaged and/or wild, related species (Ellstrand, 1988; Tiedje, et al. 1989; National Research Council [NRC], 1990). A trait associated with a transgenic gene (for example, stress tolerance) may be selectively advantageous in the wild (Colwell, et al. 1985), and may be more responsive to selection than a genetically correlated, quantitative trait (Ellstrand, 1988). Additionally, and more specifically, concerns have been raised that the escape of transgenic genes for certain traits, such as herbicide resistance, will be particularly deleterious because escape might result in the production of an over-competitive, herbicide-resistant weed (Colwell, 1985; NRC, 1990; Goldberg, et al. 1990). There have been notable objections to concerns that plants modified with recombinant DNA technology are intrinsically different than those modified by traditional breeding methods. After all, crop breeders have introduced genetically modified plants into the environment countless times without serious deleterious effects (Brill, 1985a,b). But despite the controversy, there has to date been little research directly aimed at risk assessment.

We have been asked to address several issues associated with the risk assessment of field releases of genetically engineered brassicas. When risk concerns the escape of genes, through pollen movement, into populations of wild relatives, it is necessary to identify whether pollen from the genetically modified crop can hybridize with wild relatives, and whether any of those wild relatives occur nearby (Keeler and Turner, 1990; Ellstrand and Hoffman, 1990). *Brassica* has been identified as a high risk crop for field releases because it freely hybridizes with commonly occurring weedy relatives (Keeler and Turner, 1990), and because it is grown in many parts of the

world as a seed crop. Furthermore, brassicas are undergoing genetic engineering for one of the most politically volatile traits—herbicide resistance. Thus, risk assessment for genetically engineered *Brassica* is of utmost importance.

Gene escape, or spread, incorporates the dual processes of gene flow, or the movement of genes over a distance, and selection on that gene. Our research concerning gene spread is twofold: first, we have utilized the theories of Weinberger (1978) and Shigesada, et al. (1987) to identify parameters that can define gene spread over an array of plant spatial distributions and selection regimes; and second, we have initiated field experiments that will allow us to test our models as useful tools in the prediction of gene spread (Manasse and Kareiva, 1990; Kareiva, et al., 1991).

Very briefly, from Weinberger's theory we know that gene spread can be summarized by the maximum velocity at which a gene increases in frequency over temporal and spatial scales, and that velocity can be explicitly related to rates of selection and dispersal. That is, the maximum rate of spread is a function of mean gene dispersal distance and the relative fitness of the gene in question compared to its corresponding allele in a wild population. Monte Carlo simulations have shown that within a homogenous environment, Weinberger's equations give a very good prediction of gene spread (Manasse and Kareiva, 1990). But in a natural ecosystem, the assumption of a homogenous environment may not hold.

Shigesada, et al. (1987) have analyzed equations in which dispersal rates and selection intensity are allowed to vary periodically in space. In this formulation, the rate of spread is a function of the harmonic mean of dispersal rates, and the arithmetic mean of relative fitness. Monte Carlo simulations of these equations have shown that when the arithmetic mean fitness varies over only one order of magnitude (from 1 to 10), the rate of spread can be predicted with only a function of the harmonic mean of dispersal rate (Karieva, et al., 1991). Caution must be taken in the use of these equations. It is not only the case that relative fitnesses can vary over greater orders of magnitude (one need only to imagine the performance of a herbicide-resistant brassica and a non-herbicide-resistant brassica in the presence of a herbicide). Additionally, the parameters used in these functions must be evaluated with empirical data, particularly if any real assessment of risk is to be made.

We are performing field experiments to examine the extent to which spatial variation influences mean gene dispersal distance. We are not field-testing genetically engineered plants, but we are using a system that does examine the spread of genes from an agronomic species of *Brassica campestris* into a wild, weedy *B. campestris* with the use of a suppressor for anthocyanin (courtesy of P. Williams) that we have bred into the wild *B. campestris*. The suppressor truncates expression of anthocyanin into a Mendelian trait. We follow anthocyanin as it travels out from centrally placed agronomic *B. campestris* (flowering purple pak choi, courtesy of Sakata Seed). Thus, our system closely mimics that of a genetically engineered plant and a wild relative.

As is the case with other studies that have examined the process of gene flow within one generation (Crane and Mather, 1943; Handel, 1982; Schaal, 1908), we have found that gene flow can be reliably modeled with an exponential probability distribution function. The mean of this distribution will vary with different spatial arrays. Notably, we have found that mean gene dispersal distance increases as inter-patch distance increases (Kareiva, et al., 1991). In practical terms, these results imply that absolute isolation distance is not very useful in predicting gene spread in brassica, since a pollinator will travel over very long distances to a clump of attractive flowers. This result also implies that it will be far more useful to contain pollinators by planting large border areas of alternative sources of pollen. When pollinators leave a plant with genetically engineered pollen, it can be deposited on an incompatible species.

The theory described here also requires knowledge of selection. Therefore, we would like to know the relative fitness of a genetically engineered plant in comparison to its wild relative under a large variety of ecological conditions. Our research does not specifically address this issue. We are conducting long-term field experiments in which we combine selection and gene flow in order to test whether our model can accurately predict spread.

We have outlined a "simple" scheme for predicting risk of escape of genetically engineered plants, requiring information of gene flow within a particular spatial array for a given plant, and the relative fitness of that plant compared to a pollen recipient. This information is not easy to obtain because predictions of risk will vary among planting designs and among plants with differing relative fitnesses. However, the risk of creating an ecologically deleterious plant needs to be minimized. The costs of doing nothing may in the long run be far greater than the current cost of parameter estimation for risk analysis.

References:

Brill, W. J. 1985a. Safety concerns and genetic engineering in agriculture. Science 227:381–384.

Brill, W. J. 1985b. Genetic engineering in agriculture-reply. Science 229:115–117.

Colwell, R. K., Norse, E. A., Pimentel, D., Sharples, F. E., and D. Simberloff. 1985. Genetic engineering in agriculture. Science 229:111–112.

Crane, M. B., and K. Mather. 1943. The natural cross-pollination of crop plants with particular reference to the radish. Ann. Appl. Biol. 30:301–308.

Ellstrand, N. C. 1988. Pollen as a vehicle for the escape of engineered genes? TREE 6:S30–S32.

Ellstrand, N. C., and C. A. Hoffman. 1990. Hybridization as an avenue for the escape of engineered genes. Bioscience 40:438–442.

Goldburg, R., Rissler, J., Shand, H., and C. Hassebrook. 1990. Biotechnology's Bitter Harvest: Herbicide-Tolerant Crops and the Threat to Sustainable Agriculture. The Biotechnology Working Group.

Handel, S. 1982. Dynamics of gene flow in an experimental population of *Cucumis melo*. Am. J. Bot. 69:1538–1546.

Kareiva, P., Manasse, R. S., Klepetka, B., and W. Morris. Melding models and field data to predict the rate at which novel genes will invade and spread through plant populations. Lecture Notes in Biomathematics. (In preparation).

Keeler, K. H., and C. E. Turner. 1990. On avoiding weed problems from genetically engineered crops by avoiding plants with weedy wild relatives. In: Risk assessment in genetic engineering: environmental release of organisms. M. Levin and H. Strauss, ed. McGraw-Hill, New York.

Manasse, R. S., and P. Kareiva. 1990. Quantitative approaches to questions about the spread of recombinant genes or recombinant organisms. In: Assessing Risks of Biotechnology, L. Ginzburg, ed. Butterworth, N.Y.

National Academy of Sciences. 1989. Field testing genetically modified organisms. National Academy Press, Washington.

Schaal, B. 1980. Measurement of gene flow in *Lupinus texensis*. Nature 284: 450–451.

Shigesada, N., Kawasaki, K., and E. Teramoto. 1987. The speeds of travelling frontal waves in heterogeneous environments. Lec. Notes in Biomathematics 71:88–97.

Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., and P. Regal. 1989. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. Ecology 70:298–315.

Weinberger, H. F. 1978. Asymptomatic behavior of a model in population genetics. Lecture Notes in Mathematics 648:47–96.

Paper 9
Genetically Engineered Crucifers in the Field

Thomas Mitchell-Olds
Division of Biological Sciences, University of Montana

Brassica Biology

Brassica campestris has been naturalized as a common weed in large areas of North America. Most *B. campestris* crops are self-incompatible, and pollen transfer often occurs over moderate to long distances. If transgenic *B. campestris* crops are grown in these areas, then gene escape into weed populations of *B. campestris* is virtually certain to occur. If introduced genes increase fitness, then genes are expected to spread. There is strong potential for problems with transgenic weeds.

The *oleracea* and *campestris* genomes are closely related, and have produced the allotetraploid species *B. napus, B. oleracea* and *B. napus* have not been successful as introduced weeds in North America. *B. napus* has been reported to be a weed problem in Britain, even in closed, semipermanent habitats such as railroad right-of-ways. Naturalized populations of *B. oleracea* have been reported in some areas of Britain and California. These data indicate that *B. oleracea* and *B. napus* may have the potential to become escaped weeds in some environments. *B. oleracea* crops are usually self-incompatible, while *B. napus* is self-compatible, but partially outcrossing. Gene transfer from self-compatible crops to conspecific weeds would be expected to occur at reduced (but nonzero) rates. Data on gene flow from *B. napus* into *B. campestris* indicate strong potential for gene flow from genetically engineered *B. napus* to weedy *B. campestris*. It is likely that large-scale production of transgenic *Brassica* crops will result in escape of engineered genes.

Transgenic Crop Improvement

Several types of genetically engineered crucifers are being developed, including insect and viral resistance, herbicide resistance, antisense male sterility, and alterations of biochemical composition. Risk assessment must determine whether these changes will increase or decrease the fitness of escaped genes in weedy environments. Fitness consequences of introduced genes may be dependent on the external environment or the genetic background. Genetic modifiers may alter fitness consequences of engineered genes in some genetic backgrounds. Results from a few field trials should not be extrapolated to blanket conclusions regarding safety or risk.

Insect and viral resistance is expected to increase plant fitness. Engineered resistance to multiple insect pests is expected. Such changes have great potential for creating noxious transgenic weeds. Field production of insect- and pathogen-resistant transgenic crops requires great caution, especially if resistance genes have large effects on the level or spectrum of resistance.

Herbicide resistance would increase fitness in agricultural fields. The fitness of herbicide resistant plants in herbicide-free environments is an important, unanswered question. The same safety issues are likely to apply to herbicide-resistant cultivars obtained via conventional genetic means. Environmentalists suggest that herbicide-resistant crops will greatly increase herbicide usage. Alternatively, herbicide-resistant canola may permit agronomic methods that could reduce levels of soil erosion. Should these effects be considered in regulatory decisions?

Transgenic male sterility will be of great value in crop breeding, and is unlikely to escape through pollen. It is possible that transgenic male sterile plants may increase allocation to seed production. This might increase yield, but its influence on weediness must also be considered. If male sterility genes have reduced fitness in weed populations, then release of transgenic male sterility should be safe.

Several approaches to changing biochemical composition are being researched (e.g., altered composition of glucosinolates, oils, or seed storage proteins). The fitness consequences of such changes need to be assessed. If fitness is substantially reduced by these changes, then they would not be expected to spread in the wild.

Potential for Gene Escape

In large-scale transgenic plots (e.g., commercial crop production) there will be many opportunities for gene escape. Even

with safeguards, these risks will be non-zero. In the long run, genes will escape. If they increase fitness in weedy environments, then they will spread.

Escape is very likely in *B. campestris,* due to predominant outcrossing and weedy conspecifics. Genes in self-pollinating *B. napus* are likely to escape via pollen at a reduced rate, but volunteer seeds are likely to escape. Again, if engineered plants have increased fitness then they may escape and spread. Conclusions regarding safety of transgenic crucifers require data showing that engineered plants (or transgenic hybrid weeds) have reduced fitness in weedy populations. (Such studies may be done realistically in artificial weed populations in temporary screenhouses.)

Data to document safety of transgenic plants requires close collaboration between ecologists, population geneticists, and biotechnologists. While such studies are not cheap, the consequences of a noxious transgenic weed would be far more expensive.

Unanswered Questions

1) Why is *B. napus* not a weed? How close is it to the "weediness threshold?" The answer may vary in different regions. These questions can be addressed by analysis of natural selection on *B. napus* in weedy environments.
2) How serious is herbivory or disease in weedy brassica populations? Could *B. napus* or *B. oleracea* become weeds if they were protected from most herbivores or pathogens? Would weedy *B. campestris* become more noxious if protected from pests? These questions can be examined by manipulative experiments, such as measurements of brassica weed persistence with insects present or excluded.
3) Is there a physiological cost to herbicide resistance? What is the fitness of herbicide-resistant genes in weedy, unsprayed environments? This can be addressed by development of resistant and susceptible near isogenic lines using RFLP maps.
4) What is the fitness of crop-weed hybrid plants growing in a weedy environment? Conventional wisdom suggests that these hybrids have reduced fitness. However, more detailed studies are needed.

Paper 10
Environmental Consequences of Gene Transfer in Oilseed Crucifers and Ecological Safeguards

David Pimentel
Dept. of Entomology and Section of Ecology and Systematics, Cornell University

Genetic engineering technology offers many opportunities for improving oilseed crucifers in the United States. The rapid transfer of new genetic traits from other plant types into the oilseed crucifers will enhance oil production and quality of the oil produced. Thus, the potential benefits of this new research technology is high.

Although there are potential benefits associated with the genetic engineering technology, potential risks also exist. A brief assessment of the potential risks of deliberately releasing genetically engineered oilseed crucifers into the environment is made. Some ecological safeguards are also suggested to minimize the environmental risks associated with these releases.

When genes are introduced into plants like the oilseed crucifers, there is always the possibility that these genes may be exchanged between closely related plants in nature. For example, important weed species have originated through the hybridization of two intrageneric species, such as the crosses of *Raphanus raphanistrum* x *R. sativus* (radish, a crucifer) and *Sorghum halepense* (Johnson grass) x *S. bicolor* (sorghum corn) (Colwell et al., 1986).

Several major weed species in agriculture are crucifers. Therefore, if new genetic material is introduced into commercial crucifers, there is the clear potential for the genetic material to be transferred to weed species in nature. If such a transfer occurred, it is impossible to predict what the risks would be to agriculture and the environment.

We know from past experience that when we have intentionally introduced foreign plants into the United States as beneficial crops some of these crops have in themselves become weeds. Genetic similarities betwen many crops and weeds are evident from the fact that 11 of the 18 most serious weeds of the world are crops in other regions of the globe (Colwell et al., 1985). In the United States, for example, of a total of 5800 introduced crops, 128 species of agricultural and ornamental plants have become pest weeds (Pimentel et al., 1989).

Accuracy in predicting the ecological effects of releasing genetically engineered organisms, like the oilseed crucifers, depends on the organism, the type of genetic information introduced, the particular environment into which it is released, and the availability of detailed ecological information. Clearly, the more ecological information that is available, the better position we are in to predict potential problems. However, there is no set of protocols that will allow us to predict with 100% accuracy the impact of released genetically altered organisms on agriculture and the environment.

References:

Colwell, R. K., Barnthouse, L. W., Dobson, A., Taub, F., and R. Wetzler. 1986. Letter to the Office of Science and Technology Policy, Executive Office of the President, 9/24/86.

Colwell, R. K., Norse, E. A., Pimentel, D., Sharples, F. E., and D. Simberloff. 1985. Letter to the editor on genetic engineering in agriculture. Science 229:111–112.

Pimentel, D., Hunter, M. S., LaGro, J. A., Efroymson, R. A., Landers, J. C., Mervis, F. T., McCarthy, C. A., and A. E. Boyd. 1989. Benefits and risks of genetic engineering in agriculture. BioScience 39:606–614.

Paper 11
Talking Points

Jane Rissler
National Wildlife Federation

Background

i) Oilseed crucifers *(Brassica napus)* are likely to be engineered soon to achieve some or all of the following characteristics: herbicide-tolerance, virus (and other pathogen) resistance, insect resistance, and modified nutrient composition.
ii) Oilseed crucifers have wild and weedy relatives in the United States with which they can hybridize.
iii) No transgenic oilseed crucifers have begun environmental testing under a U.S. Federal regulatory program.
iv) The major environmental risk concern posed by small-scale and commercial uses of transgenic oilseed crucifers is the potential for flow to and expression of engineered genes in populations of wild and weedy relatives.
v) Potential hazards if certain engineered genes are transferred from transgenic oilseed crucifers to and expressed in wild and weedy relatives include the following:
   a) exacerbating agricultural weed-control problems with weedy crucifers expressing genes for herbicide-tolerance, disease- and virus-resistance from transgenic crucifers;
   b) exacerbating erosion of genetic diversity by displacing native populations with those carrying advantageous genes from transgenic insect-, disease-, virus-, and drought-resistant oilseed crucifers[1]; and
   c) contributing to rapid evolution of pests resistant to pest-resistance genes. For example, expression of disease-resistance genes in populations of wild crucifers may increase selection pressure for pathogen evolution of resistance to the genes.

Controlling Risks of Small- and Large-Scale Tests and Commercial Uses of Transgenic Oilseed Crucifers

Risk, in simplest terms, is the product of the probabilities of occurrence of exposure and hazard. Risk may be reduced by reducing exposures and/or hazards significantly.

Small-scale tests. Small-scale field tests of transgenic crop plants, thus far, have relied primarily on the capacity to reduce exposure to control risk. Experimenters have significantly reduced the probability of exposure through such means as isolation, physical confinement, chemical controls, and cultural practices while they monitored agronomic traits and potential hazards, particularly those related to gene flow.

Similar approaches will likely be used with the initial small-scale field tests of oilseed crucifers. It will be more difficult to achieve exposure control with oilseed crucifers than with some other plants because of the high likelihood of crucifer crop/wild relative interactions.

Large-scale tests and commercial uses. Wild and weedy relatives of domesticated *B. napus* are so common that gene transfer to relatives is a near certainty at commercial-scale use[2]. In other words, producers should generally assume that wild and weedy crucifers will grow in the same areas where oilseed crucifers can be successfully cultivated. Exposure controls, particularly isolation of test plots and physical, chemical, and cultural methods, are not generally adaptable to large- and commercial-scale uses.

Safety of large-scale and commercial uses of transgenic oilseed crucifers depends either on developing methods to prevent gene transfer or introducing only those transgenic crucifers for which gene flow and expression in weedy and wild relatives is acceptable.

For transgenic crucifers used only for their vegetative parts, gene transfer could be prevented by introducing biological controls, for example, that prevent flowering or seed set. In transgenic crucifers valued for their seeds, these biological controls are meaningless.

Unless researchers are able to prevent gene transfer to wild and weedy relatives by other methods, certain transgenic oilseed crucifers should not be tested at large-scale or developed for commercialization.. These are transgenic oilseed crucifers to which genes have been added for traits that would provide adaptive advantage to wild and weedy relatives. These include genes for insect-, disease-, virus-, herbicide-, and drought-resistance.

Questions Regarding Regulatory Policies Governing Commercialization of Transgenic Crops

i) Should development of the following categories of transgenic oilseed crucifers be prohibited: insect-, disease-, virus-, herbicide-, and drought-resistance?
ii) Assuming that the Federal Plant Pest Act will be used to regulate transgenic oilseed crucifers, what will be the Animal and Plant Health Inspection Service (APHIS) regulatory process leading to commercial-scale uses?
iii) Once the United States Department of Agriculture (USDA) has concluded that large-scale or commercial uses of oilseed crucifers pose no plant pest threats, will APHIS have the legal authority to impose any requirements upon the use of the transgenic crops, e.g., isolation or monitoring requirements?
iv) What will be required to assess the long-term, cumulative effects of hundreds of large-scale and commercial applications of transgenic oilseed crucifers? Can those data be generated in small-scale tests?

---

[1] In contrast to many conventionally bred traits that do not provide wild plants an adaptive advantage in the environment (e.g., dwarfness), traits that are the focus of genetic engineering are likely to provide an advantage (e.g., disease- and insect-resistance).

[2] For transgenic crops lacking wild relatives with which they can hybridize in the United States (e.g., corn, tobacco), the probability of exposure leading to gene flow to wild relatives is zero.

v) Can monitoring protocols be developed to measure rates and impacts of hybridization between the crops and their wild and weedy relatives?
vi) Given that some of these crops will be used for human consumption and some may be pesticidal, what are the roles of the Food and Drug Administration and the Environmental Protection Agency in the regulatory oversight of these crops?

References:

Crisp, P. 1976. Trends in the breeding and cultivation of cruciferous crops. In: The Biology and Chemistry of the *Cruciferae*. J. G. Vaughan, A. J. Macleod, and B. M. G. Jones (eds.). Academic Press, London.

Doebley, J. 1990. Molecular evidence for gene flow among *Zea* species. BioScience 40:443–448.

Ellstrand, N. C., and C. A. Hoffman. 1990. Hybridization as an avenue of escape for engineered genes. BioScience 40:438–442.

Gould, F. 1988. Evolutionary biology and genetically engineered crops. BioScience 38:26–33.

Hoffman, C. A. 1990. Ecological risks of genetic engineering of crop plants. BioScience 40:434–437.

Hauptli, H., Newell, N., and R. Goodman. 1985. Genetically engineered plants: environmental issues. Bio/Technology 3:437–442.

Keystone Center. 1990. Panel 5: environmental issues. Proceedings of Transgenic Plant Conference. U. S. Government Printing Office, Washington.

Office of Agricultural Biotechnology. September 11, 1990. Draft. Guidelines for assessing the environmental impact of research involving the planned introduction into the environment of organisms with deliberately modified hereditary traits. USDA, Washington.

Wilson, H. D. 1990. Gene flow in squash species. BioScience 40:449–455.

Yarnell, S. H. 1956. Cytogenetics of the vegetable crops. II. Crucifers. The Botanical Review 22:81–166.

Paper 12
Biosafety Considerations for Transgenic Rapeseed

Matti Sovero, Donna Mitten, and Keith Redenbaugh
Calgene, Inc., California

Introduction

The objective of this article is to address weediness and crop safety questions about field trials, product development, and commercialization of genetically engineered rapeseed (*Brassica napus* L.). The approach of this document is to discuss issues and concerns specifically about confined field trials of transgenic rapeseed. Many of these issues will impact product development and commercialization. However, at this time, the primary focus is on confined field trials and the assessment of utility and safety of selected transgenes.

It is the intent of the biotechnology industry to develop useful crops using genetic engineering that have benefit to farmers, processors, the food industry, consumers, and society in general. The crops may be engineered to contain transgenes that reduce saturates in edible oils of rapeseed thereby benefiting consumers directly by supplying healthier oils. Another improvement directly benefiting consumers is improved taste of fruits and vegetables (such as with tomatoes). The transgenes may modify chemical components of oilseed crops like rapeseed thereby providing speciality products for industrial uses such as lubricants and detergents. Or the genes may alter structural components of the plant (such as lignin in trees) simplifying its processing thereby benefiting manufacturers as well as society by the use of lower amounts of processing chemicals or less dangerous ones. Other genes may safen the crop (such as cotton) to a particular herbicide that degrades rapidly in the soil thereby benefiting farmers and society (again) by the use of less toxic and persistent herbicides.

Genetically engineered rapeseed has been evaluated in the laboratory and greenhouse, and in field tests in Canada. The next step is conducting limited field trials in the United States under specific guidelines that minimize the risk of escape of the transgenes whether by outcrossing (to weedy relatives or adjacent rapeseed) or escape of the transgenic rapeseed. During this step, the safety of any particular transgene will be thoroughly assessed in terms of its effect on humans, animals, and the environment. Commercialization will occur only if the transgene provides a benefit with acceptable risk. The objective of the biotechnology industry is clearly to commercialize transgenic crops that have proven benefit and safety.

Discussion of Critical Issues for Confined Field Trials of Genetically Engineered Rapeseed

Potential for Gene Escape:

1. Pollen transfer out of test plots.

Outcrossing. Rapeseed *(B. napus)* is a partially cross-breeding crop with typical outcrossing of 35%. The outcrossing ratio depends on the availability of insect pollinators (honey and bumble bees, particularly), weather and the genotype of the crop. Ratios of 5–95% have been observed (Olsson, 1960). Wind is not an effective rapeseed pollinator (Mesquida et al., 1979) and has impact in pollination mainly for enhancing self pollination and cross pollination via direct physical contact between adjacent plants.

Rapeseed readily crosses with other types of *B. napus*, including fodder rape and rutabaga. It can be artificially crossed with most other *Brassica* species (for reference see Davey, [1959]), especially with the aid of embryo rescue techniques. Spontaneous crossing is, however, extremely unlikely due to differences in blooming time, inhibition of pollen tube growth in inter-specific pollinations (Röbbelen, 1960), and disturbed endosperm development (Eenink, 1975).

B. napus is, however, relatively easy to cross with *B. rapa* (syn. *B. campestris;* Thompson, 1983) and spontaneous crosses have been observed where the two species have been grown adjacent to each other. The hybrids are fertile and can bridge the transfer of the A genome genes to either direction. *B. rapa* includes cultivated types such as turnip rape, turnips and Chinese cabbage. Wild or naturalized *B. rapa* is known as bird rape, wild turnip or field mustard. This weed can occasionally be found in most parts of the United States and can be a problem in parts of the Southeast.

Rapeseed becoming a weed. This is currently a commercial issue because rapeseed does have weedy characteristics. In areas with rapeseed growing history (such as Canada), volunteer rape is a weed in rape fields especially where new rapeseed types are being introduced since there are no herbicides to cull weedy rape from crop rape. Current agricultural practices to control rapeseed weediness are 1) crop rotation using selective herbicides, 2) use of herbicide resistance in rapeseed developed using classical breeding (e.g. Atrazine-resistant rape), and 3) isolation of production fields of different rapeseed types (e.g. low vs high erucic acid rapeseed varieties). New varieties developed using genetic engineering will be grown using standard agricultural practices.

For confined release field trials, escape of the transgenes to weedy species and volunteer rapeseed can be prevented using agricultural practices used for production of foundation seed, plus adequate monitoring. A strategy is to have a sufficient isolation barrier free from rape (such as inside a field of winter wheat, soybeans or other appropriate crop) and then maintain the trial site only free of brassica for four years to insure that all volunteers are destroyed. A controlled field release would consist of the trial, and isolation barrier of no rapeseed which would be maintained free of wild turnip or naturalized rape.

2. Survival of material outside test plots.

The transgenes could be transferred outside the test plots by outcrossing with wild turnip and naturalized rape, if there is not a sufficient isolation distance. Such material would continue to disperse or to increase if there is selection for the gene. If selection is against the gene, then the gene will disappear quickly. If there is no selection, then one of two outcomes are possible: with only one or a few instances of outcrossing, the transgene probably will not spread; with large numbers of outcrossing, then the transgene may be locally established. For interspecific crosses (with wild turnip), the transgene will probably disappear if it is neutral or due to lower fitness of the interspecific hybrid and its aneuploid progeny.

3. Incorporation into gene banks.

There is a very low probability that escaped genetic material would be incorporated into gene banks and germplasm stocks. Gene bank and germplasm grow-outs are done in isolation to ensure the purity of the stock. This is done to prevent contamination with any source of contaminating germplasm, whether it is weedy relatives, commercial cultivars, or genetically engineered material. Just as growers do not plant in areas of gene bank or germplasm stock grow-outs, genetically engineered material would also not be grown in these areas.

4. Potential release due to natural disasters.

There is always a very small probability that a natural disaster such as a tornado, hurricane or flood could increase the potential for gene escape. This is probably possible only in the case when the seed is mature and is scattered over a given area by the natural disaster. For such an event, the surrounding isolation area would be monitored for volunteers to identify the severity of seed movement. Pollen would not survive the disaster or if it did, any receptive flowers would be destroyed.

Environmental Consequences of Gene Escape:

1. Types of genes inserted.

A number of transgenes are of interest for rapeseed variety improvement. For the purpose of this paper, our focus is on genes responsible for oil quality, for both food and industrial oils.

2. Environmental issues for each gene class.

Genes modifying oils for industrial and nutritional qualities and genes for antibiotic resistance are not expected to increase the fitness of the transgenic plants, thereby enhancing their competitive ability. In all likelihood, the genes will have either a neutral or slightly negative effect on fitness of any released plant and therefore not be maintained in the population. Other genes that have a neutral or negative effect on plant vigor and hardiness will also not persist.

Another class of genes is that which increases plant fitness. This can be separated into two sub-classes: genes that are maintained through artificial selection such as herbicide resistance and genes maintained through natural selection such as disease resistance. For both sub-classes, the principal problem will not be with weedy relatives, but with wild rape growing in rape fields. If the wild rape contains herbicide or disease resistance genes (regardless of the source, genetically engineered or via classical breeding), there will be selective pressure for these genotypes to survive and propagate.

Herbicide resistance in weedy rape will be a problem only where rape is grown, but is manageable. Crop rotation and good agricultural crop practices should eliminate any such wild rape.

Insect and disease resistance genes could have an impact such as increasing the fitness of wild turnip. This is the only weed that has a likely potential for crossing with genetically engineered rape in the United States. As with herbicide resistance, good agricultural practices should result in elimination of insect and disease resistant volunteer rape in rape production fields. For example, volunteer rape and wild turnip are easy to control in cereals. They are a problem in rapeseed and possibly in sugarbeet, but can be controlled using appropriate crop rotation. There will also be a need to have weed control of volunteer rape and wild turnip regardless of presence of transgenes.

The question remains, "Will transfer of insect and disease resistance genes enhance weediness of rape and wild turnip outside rape production fields?" The answer is probably not. Genetically engineered plants will contain only a few, highly specific genes for insect and disease resistance. Although of

selective advantage, such engineered genes are directly analogous to insect and disease resistance genes bred into numerous crops using classical breeding techniques. Classical breeding methods have produced cultivars that contain genes that increase survival and plant hardiness, but such resultant cultivars, though they have undoubtedly crossed with weedy relatives or had weediness potential themselves, have not resulted in increased weediness problems.

3. Environmental effects from genetic engineering.

Gene pools—there should be no environmental effect as long as the gene pools are kept separate. As with any rapeseed germplasm stock, isolation is essential for maintaining purity.

No environmental effects are expected from modification of agronomic traits.

Weediness was discussed above. If genetically engineered rapeseed becomes established as a weed in subsequent rapeseed fields, then any industrial oil or nutritional quality modifications in the weedy rapeseed could contaminate the field plantings, altering overall quality of the rapeseed crop. This is currently the situation for classically bred rapeseed varieties such as low and high erucic acid rapeseed. These varieties are grown and managed separately using good agricultural practices. Contamination may also result from uncontrolled seed production in sub-standard conditions. It is, however, to be noted that these problems are not different from those associated with the production of different types of conventionally bred rapeseed varieties.

For crop production, there will be a need to keep specific rapeseed cultivars apart, such as those that have been engineered (or bred classically) for industrial oil applications or for enhancing nutritional value. Modifications for both these purposes are not expected to have any detrimental environmental effects, in terms of altering the weediness characteristics of the crop.

This latter concept of decreasing the weediness potential of rapeseed has merit from a historical point-of-view. Crops that have a long history of domesticity (i.e. have undergone extensive selection or "breeding" over hundreds and thousands of years) have few weedy characteristics and are not weed problems. Examples are bean, corn and wheat. Conversely, crops that have only a recent history of selection and breeding, such as artichokes, forage grasses and grain amaranths, are considered weeds in certain environments. Highly domesticated crop plants have lost their ability to compete effectively in natural environments, as intensive breeding has deliberately eliminated undesirable, weedy traits. It can be expected that as rapeseed undergoes extensive breeding, it will also become less weedy.

4. Ranking of potential environmental effects.

Weediness is the major problem since *B. napus* has weedy characteristics, although no *Brassica* species are listed as among the world's worst weeds. As discussed above, oil and/or meal quality may also be an issue.

Physical Safeguards:

1. Minimal isolation distance.

The AOSCA Certification Handbook (Anonymous, 1971) recommends that cross pollinated rape have an isolation distance of 1320 feet (1/4 mile) from any contaminating source of pollen. For *B. napus* 200 meter distance should give adequate isolation.

2. Type of border rows.

A border of non-transgenic rape is not necessary for containment. A border of a cereal crop (such as wheat), soybeans, another appropriate crop, or fallow ground is needed surrounding the transgenic rapeseed field trial in which rape and wild turnip can be controlled as needed.

3. Physical barriers.

An alternative to the isolation border would be a cage to contain the pollen and prevent pollen movement. This is not practical for any but very small field trials and is not necessary with adequate isolation distances.

4. Termination protocols.

Rape seed is not killed by freezing and can survive in the field for several years.

Several alternatives would provide sufficient avoidance of contamination. One of the following would be necessary:

1. Disk under, summer fallow, plant winter wheat or other appropriate crop, and monitor next four years for volunteers. Spray with an appropriate herbicide. Do not plant actual transgenic field plot in rape for four years.
2. No-till or till, spring planting in soybeans, and monitor next four years for volunteers. Spray with Septor (metribuzin) or other appropriate herbicide labeled for soybean for control of rape. Do not plant actual transgenic field plot (including a 100-foot safety margin) in rape for four years.
3. No-till or till, spring planting in a crop that is not sexually compatible with rapeseed, and monitor next four years for volunteers. Spray with appropriate herbicide labeled for control of rape. Do not plant actual transgenic field plot (including a 100-foot safety margin) in rape for four years.
4. Methyl bromide field trial to destroy all plant material and seeds.

Temporal Safeguards:

Two approaches are identified to prevent flowering and pollen shed. However, neither would allow the evaluation of oil and meal quality and therefore have little utility.

1. Agronomic practices to prevent pollen transfer.

Any method that would prevent flowering while allowing for completion of the field trial could be used. This would alleviate the need for the isolation border.

2. Flowering modifications.

Two safeguards are 1) to destroy the crop before pollen is released or 2) to plant obligate winter rape in the spring to minimize or delay flowering (monitoring for flowering will be needed). Both would alleviate the need for the isolation border. However, these are not practical for any but small field trials and not necessary with adequate isolation distances.

Biological Safeguards:

As with temporal safeguards, the use of biological systems to prevent seed formation or to produce non-viable seed defeats the purpose of field evaluation of modified oils or nutritional composition. As already discussed, adequate isolation is sufficient for containing genetically engineered rapeseed without additional temporal or biological safeguards.

1. Self-compatible and male-sterile varieties.

Only complete male steriles will be useful. If 100% male sterility can be demonstrated, then an isolation border is not needed to prevent the escape of transgenes. Conversely, male sterility is not necessary with adequate isolation, as already discussed.

2. Induced sterility.

None available at present that provides complete sterility. If possible, then an isolation border is not needed.

3. Genetic factors to produce non-viable seed.

None available at present, but production of pollen that resulted in seeds with 100% non-viable embryos would be useful.

Conclusions

Proper management and seed practices apply to both classically bred and genetically engineered *B. napus*. At the present, for confined release into the environment, control standards for transgenic rapeseed must be at least as strict as those required for seed banks and germplasm stock and stricter in some areas to prevent outcrossing with wild turnip. Areas of greatest concern are the following:

- Isolation from other types of rapeseed
- Isolation from turnip, where a weediness problem exists
- Rotation requirements

An isolation distance of 200 meters around transgenic rapeseed field trials is necessary and can be maintained, for example, by sowing wheat, soybeans or other appropriate crops around the trial and then controlling rapeseed volunteers and wild turnip with appropriate herbicides or other weed control measures. After harvest, the field trial must be maintained free of rapeseed for four years, unless other measures have been taken to destroy remaining seed such as using methyl bromide fumigation.

If there is escape of genetically engineered plants or pollen, transgene proliferation will not happen in the absence of selection pressure for the gene. If positive selection pressure exists, large scale escape will still only be possible if proper rotation, crop management or seed production practices are not observed.

For general release into the environment (i.e. commercialization), the transgenes cannot be contained 100%. However, cross-pollination is not capable of causing significant contamination unless accompanied by improper seed production practices, and even then probably only if there is strong selection pressure associated with the contaminating gene. The greatest risks to the quality of the crop are contamination from volunteer rapeseed or weeds and admixture or mislabeling of the seed. In this respect, the problems are similar with those associated with production of different types of rapeseed and also with weeds such as wild mustard in the production field.

When the food and feed safety of a particular genetically engineered rapeseed is determined by the FDA, then escape of the transgenes from a food safety point-of-view will no longer be an issue.

References:

Anonymous. 1971. *AOSCA Certification Handbook, No. 23.* Association of Official Seed Certifying Agencies, Raleigh.

Davey, V. M. 1959. Cultivated Brassicae: information available to the breeder. Scottish Plant Breed. Stat. Rep. p. 23–62.

Eenink, A. 1975. Matromorphy in *Brassica oleracea* L. VI. Research on ovules, embryos and endosperms after prickle pollination. Euphytica. 24:33–43.

Mesquida, J., and M. Renard. 1979. Resultats preliminaires sur la pollinisation du colza d'hiver male-sterile et les modalities de production de semences hybrides. CETIOM Infs. Tech. 65:3–14.

Olsson, G. 1960. Species crosses within the genus *Brassica*. II. Artificial *Brassica napus* L. Hereditas. 46:351–386.

Röbbelen, G. 1960. Über die kreuzungsunverträglichkeit verschiedener *Brassica*-Arten als folge eines gehemmten pollenschlauchwachstums. Zuchter. 30:300–312.

Thompson, K. 1983. Breeding winter oilseed rape, *Brassica napus*. Adv. Appl. Biol. 7:1–104.

Paper 13

Dr. James T. Watkins
Sakata Seed America, Inc.

The development of guidelines for the safe introduction of transgenic oilseed crucifers is an interesting topic for consideration. The main oilseed crucifers, *Brassica napus* and *Brassica campestris,* have several biological characteristics that may open these crops up to greater debate and criticism than the introduction of some other transgenic crops. The oilseed crucifers are insect- and wind-polllinated crops that have wild relatives that persist in the same areas that the crops are grown. Additionally, these oilseed crops are somewhat weedy in that seed may shatter as the siliques dry or are harvested. Birds may shatter seed as they feed or carry seed from fields or plots. The persistence of seed viability in the soil for periods up to or

longer than one year also contributes to the weediness of the oilseed crucifers.

There are some safeguards such as isolation distances, barriers and changes in cultural practices that could be employed to minimize gene transfer from field-test plots. A four-kilometer isolation would be necessary from other crucifer crops of the same species and from wild or weedy relatives. This distance could be reduced if natural barriers such as mountains, hills or rivers exist. For small-scale trials, a screened cage could be used to provide a barrier to insects vectoring transgenic oilseed crucifer pollen. The screen size should have no larger openings than squares of 1.5 millimeters. A 200-meter isolation distance should be provided for screened cages to minimize wind-transferred pollen. Harvest techniques to reduce or control seed shatter and the possible use of seed glues can reduce the amount of transgenic seed that is not recovered during harvest. Bird barriers over field plots and windrows used in conjunction with bird cannons could reduce the amount of seed that is destroyed or moved by feeding birds. Crop rotation with a non-compatible crop (e.g., corn, wheat) or fallowing of the ground the following season will take care of volunteer transgenic weeds from shattered seed.

These and other safeguards will only reduce the potential and frequency for gene escape, they will not eliminate any possibility that gene escape could occur. Even the use of a male-sterility system would not completely remove the possibility of gene escape. What must be determined is if transgenic oilseed crucifers are to be introduced or tested in field plot situations, what are the environmental and economic risks.

Introduction of genetically modified plants either by classical breeding methods or by genetic transformation or the introduction of a new species into an environment all carry a certain economic and environmental risk. It is necessary in the introduction of any new or altered organism to weigh the benefits and risks of its introduction. In the case of oilseed crucifers transgenic improvements for increased oil content or better oil nutritional values seem to be least concerning. The introduction of plants with herbicide, insect or disease resistances may have the greater potential of some economic or environmental impact. To what level or what risk this impact may have on the environment and economy must be assessed on a case-by-case basis.

It seems that a prudent and methodical procedure for the introduction of transgenic oilseed crucifers should be followed. This procedure may include the submission of a statement of possible economic and environmental impact to a review board. This review board could then assess the risks of introduction and approve or disapprove the introduction of the transgenic oilseed crucifer. Guidelines for review should be determined and developed in public forums utilizing a wide array of talents from the public and private sectors.

Paper 14
Responses to Questions Prepared for Workshop

Paul H. Williams
Department of Plant Pathology, University of Wisconsin

Potential for Gene Escape (MRN=more research is needed):

1. Potential for gene escape is high (MRN)—escape is virtually inevitable.
2. Survival of gene will occur where selection pressures for survival is high—e.g., disease or pest resistance (MRN).
3. Probability for transgenic material entering germplasm resources depends on the selection pressures for survival—pathogens and pests provide selection pressures in both agricultural and non-agricultural ecosystems (MRN).
4. The possibility of "weather events" influencing gene escape does exist and depends on the species involved and the nature of the weather-related event.

Environmental Consequences of Gene Escape:

1. Yes, yes.
2. Selection pressures on pathogen populations for change in the pathogen populations are highly variable—relative fitness changes with genes (MRN). Linked or associated vulnerability is unknown for most or all newly created genotypes. Some of these linked or associated traits may benefit or be detrimental to weedy/wild brassicas (MRN).
3. Effects of introductions of new genes on the environment should be considered on a case-by-case basis taking into consideration the risk/benefit outcome prior to engaging in the research (MRN).
4. The order of concern of potential environmental effects would have to be determined on a case-by-case basis taking into account the nature of the particular phenotype that an engineered genotype was capable of expressing. Research is needed to know what the possible range of expressions of the phenotype would be if introduced into wild or domesticated forms of different species. Circumstances determining potential environmental effects of, e.g., a gene for herbicide resistance, would need to be assessed based on many criteria including the survival potential of the genes themselves in the absence of any selection pressures (MRN).

Safeguards

Physical Safeguards:

1. Isolation distances to be used in experiments determining appropriate isolation need careful research using genetic markers to test models for isolation. Since escape of experimental materials placed in the field is virtually inevitable, careful risk assessment should be done prior to release—then appropriate safeguards taken to lower those risks. "Bee distance" is generally accepted as approximately three miles. This should be more than reasonable (MRN).

2. Additional research on this subject would provide good insight. Border of non-transgenic plants would likely provide a better trap of stray pollen from transgenic plants than fallow ground, since brassica flowers are highly attractive to bees and other insects that could fly over the fallow to reach the pollen and nectar sources (MRN).
3. Cages of many designs could be used to deter gene transfer (MRN).
4. Destruction of the crop by chemical scorching of the tissues and turning under the soil would seem most suitable (MRN).
5. Physical and chemical prevention of flowering for those crops being evaluated for their vegetative parts would prevent gene transfer (MRN).

Temporal Safeguards:

1. Depending on the flowering characteristics of the particular brassica crop, planting date (irrigation in desert areas, etc.) could be used to alter flowering dates in relation to other possible crops or wild *Brassica* species that would serve as recipients of the brassica pollen (MRN).
2. Experimental plants requiring vernalization to induce flowering could be vernalized either physically (cold treatment prior to planting in the field) or chemically (e.g., using gibberellin, etc.), thus placing them out of flowering synchrony with surrounding plants (MRN).

Biological Safeguards:

1. Breeders can develop a number of traits through selection and/or genetic engineering that could reduce the potential for gene flow from transgenic plants. Examples of such traits would be male sterility, self compatibility, cleistogamy, reduced nectar function, apetally, apomixis, parthenocarpy, etc. (MRN).
2. Yes.
3. Yes. There are various gametic and embryonic lethals that would safen transgenic plants. These could be identified and incorporated into released plants.

Workshop Participants

MaryAnne Arnoldo
Allelix Crop Technologies
6850 Goreway Drive
Mississauga, ON L4V 1P1
Canada

Hedwig S. Boerrigter
Royal Sluis
PO Box 22
Enkhuizen 1600AA
Netherlands

Anne-Christine Bonfils
Plant Research Center
Agriculture Canada, Bldg. 21
Ottawa, ON K1A OC6
Canada

Roxanne Broadway
Cornell University
Dept. of Entomology
Geneva, NY 14456-0462
USA

Murray Bulger
University of British Columbia
6660 N.W. Marine Drive
Vancouver, BC V6T 1X2
Canada

Dennis Chamberlain
American Pedigreed Seed Co.
7664 Moore Road
Memphis, TN 38120
USA

Anne Marie Chevre
628 Rue Papu
Rennes, 35000
France

Jane Harris Cramer
Agrigenetics
5640 East Buckeye Road
Madison, WI 53716
USA

Mary Cristey
DSIR Crop Research
Lincoln Private Bag
Christchurch,
New Zealand

Philip Dale
John Innes Center
Cambridge Laboratory
Norwich, Norfolk
United Kingdom

Elizabeth Davies
Nickerson International Seed Co.
Cambridge Sci. Park, Milton Rd.
Cambridge CB4 4GZ
United Kingdom

Regine Delourme
I.N.R.A.
Stn. D'Amelioration Des Plantes
BP 29, LeRheu 35650
France

Louise Duke
Agriculture Canada
Rm. 113 DW Nearby Bldg.
Ottawa, ON K1A 0C6
Canada

Lars Goran Engqvist
Svalof AB
Resistance Breeding Department
Svalof AB S-268-00
Sweden

Zhegong Fan
DNA Plant Technology Corp.
2611 Branch Pike
Cinnaminson, NJ 08077
USA

Jacqueline Fedele
Monsanto Co.
700 Chesterfield Village Park
St. Louis, MO 63198
USA

Emile de Gruijter
TS Seeds R&D B.V.
PO Box 263
Ag. H.I. Ambacht 3340
Netherlands

Curt Hill
Calgene, Inc.
7664 Moore Road
Memphis, TN 38120
USA

Bin Huang
Allelix Crop Technologies
6850 Goreway Drive
Mississauga, ON L4V 1P1
Canada

Kathy Hudson
1741 Hobart Street, N.W.
Washington, DC 20009
USA

John E. Hunter
The Procter & Gamble Company
P.O. Box 398707
Cincinnati, OH 45239
USA

Eric Jullien
Sanofi, Inc.
101 Park Avenue
New York, NY 10178
USA

Stephen Kresovich
USDA-ARS Plant Genetic Res. Unit
Cornell University
Geneva, NY 14456-0462
USA

Jean Labriola
DNA Plant Technology
2611 Branch Pike
Cinnaminson, NJ 08077
USA

Ulf Lagererautz
Swedish University of Agr. Science
Dept. of Plant Pathology
Uppsala S-75007
Sweden

Fred Lehle
University of Arizona
Dept. of Plant Sciences
Tucson, AZ 85721
USA

Jeff Mauvais
E. I. duPont de Nemours
Experimental Station
Wilmington, DE 19880
USA

David McCarter
Calgene, Inc.
1920 Fifth Street
Davis, CA 95616
USA

James R. McFerson
USDA-ARS Plant Genetic Res. Unit
Cornell University
Geneva, NY 14456-0462
USA

Catherine Medich
Cargill Hybrid Seeds
2600 W. Galena Blvd.
Aurora, IL 60506
USA

Sally Metz
700 Chesterfield Village Pkwy
St. Louis, MO 63198
USA

Sirithan Pairoj-Boriboon
Royal Thai Embassy
4301 Connecticut Avenue, N.W.
Suite 429
Washington, DC 20008

C.E. Palmer
University of Manitoba
Dept. of Plant Science
Winnipeg, MT R3T 2N2
Canada

Oscar H. Pearson
Cornell University
404 Forest Home Drive
Ithaca, NY 14850
USA

Mark Pieper
Ferry-Morse Seed Co.
PO Box 1010
San Juan Bautista, CA 95045
USA

John Pierce
E. I. duPont de Nemours
302 Jackson Blvd.
Wilmington, DE 19803
USA

Robert Pierce
Ferry Morse Seed Co.
PO Box 1010
San Juan Bautista, CA 95045
USA

Gerhard Rakow
Agriculture Canada
107 Science Crescent
Saskatoon, SK S7N 0X2
Canada

Keith Redenbough
Calgene, Inc.
1920 Fifth Street
Davis, CA 95616
USA

Takako Sakai
Plantech. Research Institute
1000 Kamoshida, Midori-ku
Yokohama 227
Japan

Ginette Seguin-Swartz
Agriculture Canada
107 Science Crescent
Saskatoon, SK S7N 0X2
Canada

Joao Silva-Dias
Section of Horticulture
Inst. Superior de Agronomia
Tapada da Afuoa, Lisboa 1399
Portugal

Rita Teutonico
University of Wisconsin
Dept. of Agronomy
Madison, WI 53706
USA

C. Thompson
ICI Finance PLC
PO Box 14, Northwich
Cheshire, Glasgow CW9 SRD
United Kingdom

Joann Turner
Calgene, Inc.
1920 Fifth Street
Davis, CA 95616
USA

Roland E. Voorrips
Center for Plant Breeding
PO Box 16
Wageningen 6700 AA
Netherlands